THE
AMERICAN NATURALIST

Vol. XCII July-August, 1958 No. 865

# THE RELATION OF SPORE FORMATION TO RECOMBINATION

JOHN TYLER BONNER

Department of Biology, Princeton University, Princeton, New Jersey
and the Institute of Animal Genetics, Edinburgh, Scotland.

## THE RULE

If the life cycles of many different organisms that produce spores (or some equivalent resistant stage, such as cysts) are compared it can be shown that recombination is invariably closely associated with sporulation. There are some cases where all forms of recombination appear to be lacking and these particular asexual organisms will therefore provide exceptions to this general rule. By close association it is meant that the spores, when they emerge, have previously undergone recombination (or do so upon germination) and the immediate spore products are correspondingly variable. The advantage of such a system is obvious: the possibility that new geographic environments or new seasons face the emerging spore products are excellent, and the greater the genetic variability, the greater the likelihood of having a genotype especially suited to the new external conditions. A few examples will be helpful in illustrating the point.

## EXAMPLES OF THE RULE

The unicellular alga Chlamydomonas is a good case of an organism in which the vegetative stage is haploid. The vegetative individuals may divide into two, four, or eight daughter cells within the parent wall and depending upon the species they may be liberated immediately or after a period in a non-motile palmella stage. This asexual reproduction in no way involves a resistant stage. At the onset of sexual reproduction the individual cells function as gametes and the zygotes form a thick wall and pass through a period of rest. Upon germination the zygote undergoes meiosis and produces four or more flagellated daughter cells. The genetic variability resulting from recombination will be present in these individuals that emerge from the resistant zygotes.

Volvox is a more advanced relative of Chlamydomonas and the situation is very much the same except for the fact that the vegetative individual consists of many hundreds of cells bound together in a colony. The asex-

ual reproduction is achieved by certain cells in the colony dividing and producing a daughter colony which forms as an inward pocket and escapes upon the disintegration of the mother colony. As is true of many other fresh water algae, the asexual reproduction continues throughout the summer when conditions are fairly constant and favourable for growth, but as soon as the beginnings of winter come the colonies produce gametes instead of daughter colonies. The small sperm fertilizes the large egg and the zygote rapidly develops a hard, spiny wall. This resistant cyst will settle in the mud and survive the winter; in the first warmth of spring it will swell and following meiosis and successive divisions produce a new small colony that will subsequently produce progeny by asexual budding. However, it is important to note in this case that all the products of meiosis are kept in the one initial colony. This means, therefore, that only part of the reshuffling of recombination lies in the resistant spore; two different genomes of two parents are brought together, followed by meiosis, but all the progeny of meiosis are imprisoned in the first colony. This colonial genetic heterogeneity lasts only one generation since the asexual daughter colonies come from single cells and therefore the subsequent colonies will be genetically pure cell clones. The principle that recombination is essentially carried in the resistant stage holds, although the particular life history of this unusual organism requires an asexual generation before the recombined cell progeny can be completely segregated.

One final example of a haploid organs is the bread mold Rhizopus. In heterothallic forms the haploid haphae of opposite sexes abut against each other at their growing tip. On each side numerous nuclei are cut off by a cross-wall partition and ultimately these gametangia fuse by dissolving the wall that separates them. Many of the nuclei from both parents now fuse, those that fail to pair degenerate, and at the same time the whole zygospore forms a thick horny coat which is a resistant, resting stage. Meiosis apparently occurs as the zygospore germinates and generally the first mycelium is very short producing immediately an asexual sporangium. In this case the added feature is a secondary resistant spore which is found in the sporangium. At first glance it would appear that the rule concerning the association of recombination and spore formation only applied to the zygospore, but the production of asexual spores is part of the scheme as well. As with Volvox, immediately after the germination of the zygospore, the genetically different nuclei resulting from meiosis cannot be segregated since they are held in a common mycelium. Theoretically they could be separated by individual nuclei forming a new hyphal branch and in this way starting a pure nuclear clone. But even more effective is the isolation of nuclei in small groups (two to ten nuclei per spore in Rhizopus) so that the genetic segregation is completed on a large scale in the first sporangium following the sexual events. Thousands of spores will be produced, each with different combinations of the different nuclei and some of these dif-

ferent nuclear groups have an excellent chance of finding an environment that especially suits their particular genetic constitution.

If one looks elsewhere among the phycomycetes the water mold Allomyces provides an excellent illustration of the point. In the forms which Emerson (1941, 1954) has lumped under the type Euallomyces, there is an alteration of generations in which the sporophyte and gametophyte are both large plants of equal importance. The haploid mycelium produces both male and female gametangia and the uniflagellate gametes fuse to produce a diploid mycelium. At maturity this sporophyte has two kinds of sporangia: a thin-walled type that produces asexual zoospores allowing repeated asexual generations if the conditions are favourable, and a thick-walled resistant sporangium which contains numerous nuclei that divide meiotically at germination before they are cut off into separate uniflagellate swarmers (Wilson, 1952). Each of these haploid swarmers is capable of giving rise to a new gametophyte therefore here is an immediate segregation of the products of meiosis. But the interesting feature is that the sporophyte can produce two different kinds of sporangia and that the sporangia in which recombination occurs are of the thick-walled resistant type. Emerson showed that if the latter were germinated precociously meiosis did not occur and sporophytes were produced instead of gametophytes. There appears to be a close relation between the two types of spores, but in keeping with the rule set forth here, recombination is associated with resistance.

It might be added that these facts apply equally well to the more advanced Cystogenes type of Allomyces where the gametophyte has been greatly reduced to small circular protoplasts that produce gametes directly. Of course the trend for gametophyte reduction is seen even more clearly in the evolution of the higher plants.

The principle may be readily illustrated in the higher fungi also. In ascomycetes there is recombination and segregation with the formation and dissemination of the resistant ascospores. The fact that some forms (for example, Neurospora) also produce micro and macrocondia which are to some extent resistant will be examined presently. The nuclear behavior of ascomycetes is of interest in that haploid nuclei of diverse parents may flow in one mycelium and they will fuse in pairs shortly before the meiosis preceeding ascospore formation. In the basidiomycetes only two parents may be involved; two haploid hyphae fuse to form a dikaryon in which the pairs of nuclei remain together in each cell and again wait until the last moment before fusing, just prior to the meiosis that leads to the formation of basidiospores.

The fact that mosses, ferns and the higher groups of plants also fit the rule is so obvious that the briefest summary is adequate. In the mosses the main body of the plant is haploid, fertilization takes place at the tip, the sporophyte grows as a second story and it produces the spores at its tip. Since the spores give rise to new gametophytes, sporulation and re-

combination occur together. In ferns the main plant is diploid, the gametophyte being confined to a small delicate prothallus. The prothallus produces gametes which upon fertilization gives rise to the fern proper and all along the edge of the leaves there are spore-bearing sori. These spores, as in the mosses, give rise to the gametophyte, again an association of recombination and sporulation. Beyond the ferns the gametophyte becomes so inconsequential that it eventually is never separated from the great sporophyte and we have two new innovations which bear on our argument.

One is the invention of pollen as a substitute for a motile sperm. Pollen does not possess a very thick wall, but nevertheless it is to some extent resistant. Furthermore, it certainly plays an important role in recombination, although a partially different role from that of our previous examples. Pollen carries the products of meiosis of one of the two gametes; therefore, this does not directly benefit the whole organism in producing resistant variants to face new environments. Instead the benefits are confined entirely to fertilization; it is an effective method of permitting variable male gametes to travel great distances without destruction by dessication and other environmental severeties. Since part of the success of recombination depends on cross breeding between geographically distant individuals, pollen serves as a substitute for motility, a property generally lacking in plants.

The second innovation of higher plants is the formation of a hard, resistant seed. In this case the zygote does not immediately become encysted, but first grows for a period in the protective and nutritious environment of the ovary. It is a case of prolongation of the parental care of the progeny, an evolutionary advance that is also characteristic of the higher animals, as in placentation of mammals. Eventually the developing embryo comes to a resting point and is sealed off with large food reserves ready for its external distribution. There is here a slight separation of recombination and the resistant stage, but from the point of view of the distribution of variable resistant progeny in new environments and new seasons, the mechanism is the same as was found in the algae and the fungi.

For the most part metazoa possess only one type of resistant stage which is the fertilized egg, an exact equivalent to the zygospore. In many animals the egg is in no way resistant, while in others it is conspicuously so. When it is of the resistant type then its relation to recombination is again simple and straightforward. Depending upon the animal there is variability as to how much development has occurred before the egg becomes hardened; in some cases there are simply the male and female pronuclei, while in others an embryo will be partially formed. In this it resembles the higher plants as well as in the fact that food in the form of yolk is stored along with the embryo.

A striking illustration of the principle occurs among the aphids. In numerous species of these insects the sole method of reproduction during the summer is through the viviparous production of parthenogenetic indi-

viduals. By this particular mode of specialized asexual budding the aphids produce many offspring that may immediately benefit from the plant juices that are abundant in the warm seasons of the year. As the cold weather approaches sexual males and females appear, fertilization ensues and resistant eggs that survive the winter are deposited externally on the surface of the host plant. Therefore the only time that meiosis occurs in the annual cycle of these aphids is also the only time that resistant eggs are produced, and these hatch with the new season the following year. A very similar story is to be found among the water fleas (Cladocera).

## EXCEPTIONS TO THE RULE

If we now look for exceptions to the rule the only good cases that exist are in certain asexual organisms or certain organisms which have an asexual spore or cyst stage. This is not true of all asexual organisms, for as will be shown, some have a modified kind of recombination. Even in most cases which appear to be clear-cut exceptions there are mitigating factors. In some, where one will find a resistant stage but no recombination, they nevertheless are obviously closely related to forms that have both. For instance in a parthenogenetic or self-fertilizing organisms there is little or no possibility for recombination, yet the degeneration of the normal cross-breeding is clearly secondarily derived, presumably to stabilize a successful genotype in a specific, constant environment. In other asexual organism, especially in poorly understood lower forms, one is always plagued with the doubt that perhaps these organisms are considered asexual because no one has yet demonstrated sexuality. And even in some cases where sexuality has been demonstrated, the details of the complete cycle are not clear and the relation between cysts and sexuality uncertain.

Nevertheless, it is reasonable to assume there are some organisms that are asexual and certainly cysts would be advantageous even without any recombination. Among the rhizopods some of the solutary soil amoebae such as Hartmanella have beautiful cysts yet sexuality is absent. For an organism which is merely a delicate naked protoplast, and that lives in soil which may freeze or dry out, the advantage of a resistant stage is not hard to see. This problem is undoubtedly dealt with in a similar fashion by many protozoa, bacteria and other lower forms.

## ASEXUAL ORGANISMS THAT FIT THE RULE.

In some organisms, as Haldane (1955) has pointed out, the asexual spores provide a modified kind of recombination. This fact has been recognized for some time as shown by Hansen and Smith's (1932) analysis of the handling or variation in imperfect fungi. The point is simply that if an organism contains mixtures of genetically different nuclei, which is the case for heterocaryons, then when the nuclei are separated in small spores containing one nucleus (microcondria) these spores can germinate and fuse in dif-

ferent combinations to produce new heterocaryons of different genetic constitution. This is a crude recombination without meiosis and its attendant chromosome reshuffling, although some chromosome recombination occurs in those forms which possess vegetative diploidization and mitotic reduction as described by Pontecorvo (1956). In the case of macroconidia of ascomycetes and the spores of phycomycetes such as Rhizopus where there may be more than one nucleus, there is again a possibility of this kind of recombination because chance will dictate what combination of nuclei are encased in a particular spore.

A parallel situation has recently been established in our laboratory with the cellular slime molds. There a number of amoebae stream together in a cell mass and the mass as a unit forms a fruiting structure bearing numerous uninucleate spores. M. F. Filosa (1958) has shown that this cell mass can be composed of genetically diverse cells, and this condition has been termed "heterocytosis." Upon sporulation any particular cell combination or heterocyton is segregated with the possibility that the germinating spores can recombine in new ways. Again here the resistant stage is intimately tied up with this particular type of recombination.

This argument for the cellular slime molds could conceivably apply to sponge gemmules as well. In many species of sponges, especially fresh water forms, the organism survives the winter by the production of hard resistant gemmules. These are formed by the migration of many large amoebae to collection centres, and by a series of remarkable steps this mass of archeocytes is provided with food and surrounded by a hard coat lined with spicules. Unfortunately, there is no evidence on this point, but presumably, since many individual sponges will lie side by side and fuse into one another as they cover twigs and other debris on the bottom of a pond, this aggregation of archeocytes could bring together genetically different cells from different sponges. Should this happen then the gemmule would also be a heterocyton. Since the gemmule does not segregate the cells but they together build one sponge after germination in the warm weather, the sole recombination that could theoretically take place here would be in the bringing together of divergent cells during the formation of the gemmule. Since this has not been demonstrated as yet the sponges present an intriguing hypothetical example of our rule.

## SUBSTITUTES FOR A RESISTANT STAGE.

There are other ways besides spore formation to insure that the products of recombination are made available to react with new environments. Sonneborn (1957) points out that the work of Cleveland provides an unusual example. Cleveland showed that the moulting hormone of the wood roach stimulates sexual reproduction in certain of the flagellates living in its gut. At moulting the protozoa are extruded in the fecal pellets and at the same time the eggs, which have been laid previously, begin to hatch and

take on their intestinal fauna. In this case meiotic recombination has become dependent on the endocrine balance of the host and the mechanism is devised so that the moment in their life history that the protozoa progeny enter new environments they are maximally variable. However, as Sonneborn cautions, the meiotic processes in present day roach flagellates is, according to Cleveland, so modified that effective recombination is reduced. From this Sonneborn suggests that the ideal situation with normal meiosis was ancestral when rapid evolutionary changes were in progress, and what we have today is a degeneration to reduce recombination so that the successful roach-flagellate symbiosis can be maintained in stability. From the point of view of evolution, this meiotic reduction would be acting in the same way as parthenogenesis or self-fertilization found in other static forms.

Sonneborn also points out that one of the significant differences between varieties of *Paramecium aurelia* that are outbreeders rather than inbreeders is the fact that the juvenile or pre-conjugation period is greatly extended in the outbreeders. In other words if maximum recombination is sought, as would be the case for outbreeders, then the longer the period before conjugation, the greater the possibility of the individuals finding mates from distance geographic sources. In this case only one of the advantages of spore formation is present, that of creating a delay, which increases the efficiency of cross-breeding. However, these ciliates differ from spores in that the juveniles are in no way resistant.

This now brings the final point or corollary to the rule which has been set forth here. It concerns those cases, found in particular abundance among the animals where there obviously has been rapid evolutionary progress, where the recombination mechanism is exploited to its full capacity, yet there are no resistant stages associated with the recombination. The example just cited of *Paramecium aurelia* serves to underscore the importance of the proper setting needed for recombination. The setting is defined both by the environment and the structure or habit of the organism. If the organism is of such a nature that it can exist in adult stages only during some seasons of the year, then it requires a method of preserving itself during the unfavorable seasons. If an organism is restricted geographically because of lack of motility, then a small resistant stage that can be carried large distances, is advantageous. Both these factors lead to some type of resistant stage. Many animals have managed to be active in some form throughout the year and are sufficiently motile so that they themselves can reach the new environments. In these cases, then recombination alone is adequate for selection because the more complete physiological adaptation to the changing environment and the ability to migrate have become substitutes for spore formation. In fact, if one examines the progress of animal evolution there is a constant tendency to decrease the exposure of the egg and early embryo by viviparity, placentation and extended parental care, which has the great advantage of being able to coddle,

protect and teach a more complex offspring. But recombination is no less urgently required in the breeding and selection of these advanced animals and therefore the developmental advantages gained by eliminating a resistant stage has only been possible through motility and the continued maintenance of the adult stage in adverse seasons.

ACKNOWLEDGEMENTS

I am indebted to Dr. J. Reissig and Professor C. H. Waddington of the Institute of Animal Genetics, University of Edinburgh, for reading the manuscript and providing useful suggestions. Also I should like to gratefully acknowledge Professor Ralph O. Erickson's interest and help with these ideas at an earlier stage of their development.

LITERATURE CITED

Emerson, R., 1941, An experimental study of the life cycles and taxonomy of Allomyces. Lloydia 4: 77-144.

1954, The biology of water molds, 13th Growth Symposium, Princeton University Press, pp. 171-208.

Filosa, M. F., 1958, Heterocytosis in cellular slime molds, Thesis, Princeton University.

Haldane, J. B. S., 1955, Some alternatives to sex, New Biology 19: 7-26.

Hansen, H. N. and R. F. Smith, 1932, The mechanisms of variation in imperfect fungi — *Botrytis cinerea,* Phytopathology 22: 953-964.

Pontecorvo, G., 1956, The parasexual cycle in fungi, Ann. Rev. Microbiol. 10: 393-400.

Sonneborn, T. M., 1957, Breeding systems, reproductive methods, and species problems in protozoa. From The Species Problem, ed. by E. Mayr, A.A.A.S., Washington, D. C., pp. 155-324.

Wilson, C. M., 1952, Meiosis in Allomyces. Bull. Torrey Bot. Club 79: 139-160.

Vol. XCII, No. 865 The American Naturalist July–August, 1958

# ON PHENOCOPIES, THEIR DEVELOPMENTAL PHYSIOLOGY AND GENETIC MEANING*†

WALTER LANDAUER

Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Conn.

Some inquiries in the fields of analytical teratology, experimental embryology and physiological genetics have in recent years experienced a convergence of their paths. These investigations, though often undertaken for unrelated purposes, have tended toward a common denominator in the phenomena known as "phenocopies," the experimental production, that is, of morphological modifications which bear a close resemblance to hereditary deviations from the normal phenotype. The realization that in these mimicking reactions of developing organisms we are confronted with something more important than fortuitous contingencies has heightened their interest for developmental physiology and genetical theory. Although inquiries into the origin and meaning of phenocopies are still too much in their infancy to permit final interpretations, tentative conclusions are emerging from this work and to their discussion I propose to address myself.

The phenocopy concept emerged from experiments with Drosophila in which the developmental effects of heat shocks were studied (Goldschmidt, 1935). But most recent information, stemming chiefly from work with *Drosophila melanogaster* and chicken embryos, was obtained by exposing the developing organisms to a variety of chemical agents. The more important contributions to the chemical production of phenocopies in Drosophila can be found in the publications by Rapoport (1939), Gloor (1944), Bodenstein and Abdel-Malek (1949), Sang and McDonald (1954), Bertschmann (1955), Goldschmidt and Piternick (1957 a, b), and Goldschmidt (1957). For the work on experimental phenocopies in chicken embryos reference is made to my publications on the subject (Landauer 1954, with review of earlier work; 1955-57). The principal conclusions to which this work has led are shown in the form of a tabular summary (table 1).

It is a striking fact that many of these conclusions apply equally to Drosophila and to the chicken embryo. This suggests at once that we are dealing with developmental responses which are the common property of quite unrelated animal forms. Past experience, moreover, encourages the expectation that the area of agreement will continue to broaden as further investigations proceed. Let us now examine the major generalizations and

*Extension of a brief report to be presented at the Tenth International Congress of Genetics, Montreal, Canada, August 1958.

†Based on work supported by a grant from the Association for the Aid of Crippled Children.

TABLE 1

THE PRINCIPAL GENERALIZATIONS CONCERNING PHENOCOPIES ACCORDING TO THEIR SOURCES OF DERIVATION

| Chicken | Drosophila and chicken | Drosophila |
|---|---|---|
| Plus modifiers of homologous mutants reduce response to phenocopy-inducing agents; this effect may extend to stages in which non-homologous phenocopies are produced | Stage dependence | Qualitative response differences between stocks to same teratogen |
| The same supplement may protect against similar phenocopies produced in different stages and by distinct teratogens | Quantitative dosage/effect relationship (penetrance and expressivity) | Tendency for qualitative response norm to be dominant in $F_1$ |
| The same supplement may protect against dissimilar phenocopies produced in identical or different stages by distinct teratogens | Relation to toxicity, often ill-defined (sub-lethal dosages generally required) | Heterozygosity for homologous, recessive mutant genes in some instances increases, in other cases lowers, response to phenocopy-inducing agents |
| Differential protection against (and differential potentiation of) parts of a phenocopy-syndrome | Quantitative response differences within and between stocks to same teratogen | |
| | Selection for or against response effective | |
| | Enhancement of response by homologous hereditary stock tendencies ("sporadic" variations; multifactorial mutant conditions with low penetrance) | |
| | Influence of maternal genotype, especially in early developmental stages | |

their implications from the viewpoint of developmental physiology and from that of genetics. In both respects we shall be particularly interested in determining how well the results of studies on phenocopies accord with the facts and interpretations familiar to the embryologist and the geneticist.

## PHENOCOPIES AND DEVELOPMENT

To begin with, the response of reacting organisms to phenocopy-producing chemical compounds is a function of their internal organization, and as a consequence is determined by the developmental stage attained at the time of treatment. The changes in response as a function of developmental stage may be of a qualitative nature, quite unrelated morphological effects being produced by the same agent in different stages, but within any one sensitive period one may also observe an increase to a maximum of "penetrance" and "expressivity" with a gradual decline thereafter. There is, moreover, evidence suggesting that in phenocopic syndromes protection by

appropriate supplements against the phenocopy-producing agent is the more effective the farther removed from final determination a particular part or organ is at the time of treatment (Landauer, 1957).

No more surprising than these stage relationships is the fact that the force of the external insult imposed upon developing organisms plays a decisive role in determining the result. These dosage effects are chiefly of a quantitative nature, and the fact that the dosage-effect dependence often follows a straight line bolsters our confidence that we are dealing with physiological interactions within the developing embryo. In addition, however, there may be qualitative differences, new parts of the embryo becoming involved with rising dosage.

As long as the genetic milieu is kept constant, developmental stage and dosage are the principal factors which determine the response to a given phenocopy-producing agent. With reference to these factors the value of phenocopy studies has been questioned (Hadorn, 1955) because penetrance of the induced traits often is far from complete and because expressivity and organ specificity may vary. There are, however, many phenocopy-producing compounds with which well-defined traits or syndromes can be produced with a 100 per cent incidence (e.g., sodium tetraborate in Drosophila; thallium and nicotine in chicken embryos). Moreover, to say that genes are more "reliable" in their effects than are phenocopy-producing teratogens, is at best only true if the mutant standards of comparison are those favorite traits of geneticists (actually probably the minority of mutants) which show a simple manner of transmission and expression. It is an interesting fact, on the other hand, that with none of the phenocopy-producing substances which have been used to date can a full response be obtained without using amounts with considerable toxicity. This holds true even though the relationship between teratogenic activity and toxicity varies greatly from one compound to another, and in spite of the fact that the nature of the relationship remains obscure. Some of the mutants that are copied are, of course, also lethal, but in the majority of instances the phenocopy-producing teratogens presumably have systemic effects with which the equilibrating or recuperative resources of the developing embryo cannot cope.

Our experiments with chicken embryos have demonstrated that the phenocopy-producing chemical compounds accomplish their end by interfering with definite metabolic events. This is clearly proven by the fact that specific metabolites, if given as supplements, provide protection, in some cases complete in other instances only partial, to the treated embryos (e.g., riboflavin against boric acid; tryptophan against 6-aminonicotinamide and 3-acetylpyridine; nicotinamide against insulin, sulfanilamide, eserine, etc.; L-proline, DL-lysine and some other amino acids against nicotine). The production of phenocopies may, but need not, be associated with a general retardation of growth (body size); in the case of insulin-induced rumplessness, for instance, body size is normal, but is reduced in association with

micromelia produced by treatment with sulfanilamide or 6-aminonicotinamide. The specific effects of the last two teratogens (namely, micromelia and beak defects) can be completely forestalled by providing supplementary nicotinamide. Such supplementation permits in the case of 6-aminonicotinamide the hatching of normal chicks (Landauer, 1958), whereas body size remains reduced after sulfanilamide treatment in spite of the absence of malformations (Zwilling and DeBell, 1950). These observations presumably rule out the conclusion that the specific (phenocopic) effects appear as a response of particularly sensitive parts to a general retardation of growth. It must be concluded, on the contrary, that–whatever may in some instances be responsible for reduced body size–the localized phenocopic effects occur in consequence of an interference with localized metabolic needs. For our subsequent discussion of the genetic situation it is important to note the following further features as revealed by studies with supplements: (1) in an experimentally-induced syndrome (for example, beak defects and micromelia) which in its totality represents a phenocopy of a known mutant condition, it has been possible to alleviate or forestall by supplementation one aspect (beak) without furnishing protection against the other (micromelia); contrariwise, selective exacerbation can also be accomplished. (2) In the case of teratogens which produce morphologically unrelated phenocopies when applied in different developmental stages, the same supplement will in some instances furnish protection in both stages, whereas different supplements are required in other instances. (3) Phenocopies of distinct mutants, brought about by different compounds but during one and the same developmental stage, are in some instances forestalled by identical supplements.

There is much additional evidence, particularly from experiments with those teratogens which produce results with relatively minor but constant differences, attesting to the high degree of biochemical diversification which provides the basis for many subtle dissimilarities of local responses. We know very little as yet about the biochemical nature of the reactions which are involved in the origin of phenocopies. It is true that much of the evidence from work with chicken embryos suggests that an interference with co-enzymes plays an important role. But even in this respect we must recognize that the situation is very complex indeed–as shown, for instance, by the fact that certain supplementary compounds (for example, nicotinamide or tryptophan) provide protection against a wide spectrum of apparently unrelated teratogens.

The developmental picture of phenocopies which we have presented thus far portrays a complex of predictable, if only partly-understood, conditions to which the organisms react with the production of "phenodeviants." The fact that phenodeviants appear is an expression of the incapacity of these organisms to fulfill their genotypic destiny, but in many instances (at least as far as chicken embryos are concerned) the natural equilibrating processes can be brought into balance by furnishing the proper supplements. As

is true for mimicking mutant conditions, differences in developmental details may exist between identical phenocopies produced by different means or between phenocopies and their genetic counterparts.

Until recently it was tacitly assumed (though the existence of stock differences in response had been recognized) that phenocopies are produced as unspecific reactions of the species serving as test object, that is, without reference to its particular genotypic make-up. Much evidence has now come forward, however, for the conclusion–which might indeed have been foreseen–that the phenocopy-producing reactions of developing organisms depend upon the specific genotype and its gene-controlled developmental capacities. Thus, it was found that the quantitative response to a phenocopy-producing agent may vary with the genotype and that selection in a plus or minus direction generally is effective. Furthermore, it became evident that homologous stock tendencies, that is, the presence of genes with low penetrance but similar phenotypic expression, is likely to enhance greatly the efficacy with which corresponding phenocopies can be produced.

All of the foregoing conclusions hold equally for Drosophila and for the chicken, thereby once again attesting to the fundamental nature of these relations. Qualitative differences of response to one and the same teratogen have been found between various stocks of Drosophila, and the typical response of any one stock usually was dominant in the $F_1$ generation of crosses between stocks. In chickens the maternal genotype frequently has an influence on the response or lack thereof of phenocopies induced during early stages of embryonic development and similar situations apparently occur in Drosophila (Waddington, 1957).

## CONTRASTED INTERPRETATIONS

These and still other genetic facts which have been discovered in phenocopy studies are at present open to two principal interpretations. Goldschmidt and Piternick (1957a) on the basis of their experiments with Drosophila incline to the view "that the different effects of treatment upon different genetic lines are due primarily i.e. apart from the differences in the modifier system, to the presence of sub-threshold alleles (we prefer this term to isoalleles) of the mutants which are phenocopied; and that their action is raised above the level of visible effect by the treatment." In another place Goldschmidt (1957) concludes that "the hypothesis can hardly be avoided that all phenocopies are due to a bringing into light of already present non-penetrant, subthreshold or isoallele mutants." Goldschmidt and Piternick concede, however, that this interpretation does not fit all of their data, and in a subsequent publication (Goldschmidt and Piternick, 1957b) they allude to the possibility that the effective mechanism "might be a combinstion of subthreshold isoalleles for different loci with a set of different more or less specific suppressor loci, again of different potency, the action of which is more or less canceled out by the

same treatment." The alternative interpretation of the situation, defended here, is that phenocopies represent the end-result of an interference with the activity of the gene or genes for normal or mutant phenotype, as the case may be, observed in the phenotype of the control material. We assume, furthermore, that the degree of this interference and its consequent expression will vary with the number and kind of modifying genes, associated with the locus (or loci) whose expression is modified, as they happen to exist in a particular stock.

It may be taken for granted that, by the conditions existing within the internal and external environment of an organism, the activity of every gene is attuned to definite limitations. The scope of these limits presumably varies from one gene to another. Within relatively narrow limits each gene will fulfill its physiological functions at a given rate and intensity. Beyond these limits genic activities will be modified and eventually cease or become physiologically ineffective. Interesting evidence of this nature has been provided by Glass and Plaine (1952; see also Glass, 1957) in the case of certain suppressor genes of Drosophila mutants. It is equally well established that the emergence of every trait depends on the integrated activity of many genes, if not the entire genome. It is clear, however, that not all genes have equal weight in the developmental balance that has been established during evolution. There are many genes, varying in kind and number from stock to stock, which appear to make minor contributions to the establishment of a particular trait, but which have apparently been accumulated as reinforcements against the effects of disturbing elements, whether they be mutant genes or external conditions. These are the genes that we have grown accustomed to call 'modifiers'; they are the principal elements responsible for physiological equilibration and regulatory integration during development, thereby keeping events within their "canalized" paths.

The more successful is the construction of such co-ordinating and corrective mechanisms, the less will be the probability of their being thrown out of gear by mutations or unfavorable conditions of the environment. Flaws of these mechanisms, on the other hand, may be revealed in adverse situations as genetic traits with low penetrance or by a heightened response to phenocopy-inducing agents. The greater the potential instability, the easier it becomes to suppress attainment of the phenotype which is realized under more normal conditions. There is plentiful evidence for these generalizations. Cases in point, for instance, are, in my opinion, the heightened response with phenocopies found by Goldschmidt and Pitternick, following treatment with sodium tetraborate, in Drosophila stocks which are heterozygous for an homologous, recessive mutant or which carry mutants that lack penetrance on account of the presence of plus modifiers. A similar situation was encountered in poultry with the observation that the likelihood of inducing phenocopies of rumplessness rises with the frequency of its spontaneous occurrence in a stock and that the spontane-

ous occurrence itself has a genetic basis, as shown by its contribution to increased penetrance of the mutant for recessive rumplessness. Similar facts, if not in the context of phenocopies, have been known since long. One particularly instructive example stems from work by Dobzhansky and Bridges (1928) and Dobzhansky (1930) on intersexuality in Drosophila. In their experiments with triploid intersexes they could demonstrate that (1) multiple modifying genes exist in the presence of which the sexual traits are shifted either towards maleness or towards femaleness, that (2) similar shifts can be accomplished by forces of the environment (temperature), but that (3) neither the modifiers nor the external environment have such effects in a normally-balanced sexual genotype. It is clear, therefore, that modification towards normality tends to occur in the absence of proper genic equilibration and that the same lack of gene-controlled physiological homeostasis also opens the door to greater effectiveness of phenocopy-producing agencies.

## EFFECTS OF MODIFYING GENES

All work relating to the genetic basis of phenocopy production, in Drosophila as well as in fowl, has shown that modifying genes play an important role. The question that interests us in the present context is whether the modifiers encountered in normal populations, and which tend to shift mutant conditions toward the normal phenotype, play a similar role in the development of phenocopies. With reference to the dominant mutation for rumplessness we had concluded long ago that such modifiers "are apparently constituents of the normal gene constitution, tending to promote the normal development of an important part which is especially subject to variation from different causes"; and that "they would be retained in the normal type because of their obviously favorable effect on development" (Dunn and Landauer, 1934, 1936). In these as in other investigations (for instance, Green, 1957) the observed effects of the modifiers were strictly local, but this is not universally true (see below).

We have already made reference to the parallelism existing between degree of penetrance of sporadic rumplessness and homologous response to phenocopy-inducing agents. There is little doubt that the penetrance is regulated by the residual heredity (that is, the modifiers) and is in turn correlated with susceptibility to interference from without. Still more far-reaching evidence was obtained in our experiments with the recessive "short upper beak" lethal mutation of fowl. The accumulation by selection of multiple modifying genes drastically reduced the effects of the mutant gene on beak, long bones and viability, in some instances even resulting in an overcompensation of length of the upper beak. This selected stock showed heightened resistance to the phenocopy-inducing action of insulin not only at the stage at which development of beak and long bones respond to treatment with insulin, but also at the much earlier stage when insulin

treatment produces phenocopies of rumplessness. A similar situation was found in stocks of Creeper fowl, the modifiers of the creeper condition providing a significant degree of protection against the rumplessness-inducing action of insulin (Landauer, 1946, 1947).

Such results can be understood readily on the assumption that the plus modifiers of mutant action promote normal development not only in the presence of the mutant gene but also when development is endangered by phenocopy-inducing agents, and on the more fundamental assumption that phenocopies are brought about by an interference with the activities of the gene complement responsible for normal development. I feel, on the other hand, that Goldschmidt's appeal to special genes, whether they be called sub-threshold mutants (isoalleles) or a combination of isoalleles and suppressors, places an insupportable burden on his hypothesis. From the purely genetic point of view it is difficult to accept the numerical implications of such an hypothesis; for, as the success in producing phenocopies advances, the number of isoalleles required would ever more closely approach or even exceed the number of mutant loci now known (cf. Goldschmidt, 1954).

Still greater, I believe, are the difficulties for Goldschmidt's interpretation in relation to developmental physiology. Goldschmidt has shown that a large number of different phenocopies can be produced in Drosophila by treatment of the larvae with sodium tetraborate. In assigning separate isoalleles for each of them, one must surely also assign a specific function to each. This, in turn, requires the assumption that all these independent entities respond in a similar fashion to one external agent, namely, sodium tetraborate. Such a uniform effect on *primary* gene activity is, it seems to me, very unlikely.

The quantitative aspects of the production of phenocopies present another serious obstacle to an interpretation in terms of isoalleles. In many instances one may, by applying increasing amounts of a particular teratogen, obtain a graded series of phenodeviants which leads by imperceptible steps from very slight defects to grotesque abnormalities. It is surely impossible to invoke in explanation a corresponding series of sub-threshold alleles, but there is no difficulty in interpreting these facts as an expression of a quantitative relation between the external insult and the capacity of the gene-controlled processes to fulfill their usual functions.

Any explanatory hypothesis, moreover, ought to take into account the problems posed by phenocopies of the normal phenotype, produced in mutant stocks. Examples from Drosophila experiments are the production of wild-type eyes in vermillion stock by supplementing the medium with tryptophan (Valadaresda-Costa and Jacquet, 1952) and the production of eyes with a normal facet number in Bar-eyed stock (Kaji, 1954-56) and in the mutants Glued and brown (Wahab, 1957) by supplements of ureids and acid amides. The facet development of Bar eye was also favorably influenced by supplements of nucleic acids, certain organic acids, sugars, polyalcohols and

magnesium, effectiveness of the supplements varying with residual heredity (Ogaki, 1957). In chickens phenocopies of the normal condition can be obtained in genetically polydactylous embryos as a temperature effect (Sturkie, 1943; Warren, 1944) or by treatment with insulin (Landauer, 1948). As in some of the material previously reviewed, heterozygotes respond more readily to these phenocopy-inducing agencies than do homozygotes. The expression of the polydactyly mutant may also be overcome by multiple modifiers; and in a similarly parallel way modifications in the mode of expression of polydactylism (for example, polyphalangism) can be brought about by selection or by experimental intervention. There is, in fact, no sharp boundary between phenocopies on the one hand and normal overlaps or variations of expression within the mutant range on the other; ease of phenocopy production and of genetic modification appear to spring from the same source, the degree of hereditary equilibration. As the activity of a mutant gene can be helped toward normality, so normal gene functions, it appears, can be hindered in their proper performance.

TABLE 2

THE ROLE OF 3 METABOLITES IN PROTECTING CHICKEN EMBRYOS AGAINST A SYNDROME OF MICROMELIA AND BEAK DEFECTS PRODUCED BY 3 DIFFERENT COMPOUNDS. THE + SIGN INDICATES PROTECTION, THE − SIGN FAILURE TO PROTECT

| Teratogens | Supplements | | |
|---|---|---|---|
| | Nicotinamide | Tryptophan | Riboflavin |
| Insulin | + | − | − |
| 6-aminonicotinamide | + | + | − |
| Boric acid | − | − | + |

The hypothesis that phenocopies are the result of a breakdown of physiological equilibration during complex developmental events helps us to understand some observational facts which otherwise would remain puzzling. Firstly, one would expect on our hypothesis that integrated developmental sequences, involving rate-dependent processes and/or thresholds, are more vulnerable to phenocopy-inducing agents than are those traits which are under more direct genic control (cf. Hadorn, 1955). This is, indeed, what has been found. Witness the comparative difficulty of producing phenocopies of mutations involving pigmentation. Secondly, it is a striking fact that there are instances in which closely similar phenocopy syndromes (for example, micromelia and beak defects in chicken embryos), produced in the same stock but by chemically different teratogens, can be forestalled by *different* (but not by the same) supplements. The data of table 2 are offered as an illustration. This can, on our hypothesis, be readily understood as the result of interference with two different steps in one and the same metabolic chain; in Goldschmidt's interpretation it would presumably call for postulation of separate isoalleles. Thirdly, the fact

that development of certain morphological structures is much more prone than that of others to be pushed into the direction of a phenocopy and that these same structures are particularly subject to interference by major mutations with homologous effect seems a clear indication of high vulnerability to both sources of variation on account of poor equilibration of one or more steps.

There is presumably, however, another important reason for the propensity of certain developmental processes to be channelled into the path of a phenocopy, namely, the nature of the biochemical events that follow exposure to the various teratogens. There is little doubt that many, if not all, phenocopies are brought about by interference with enzymatic processes. In work with Drosophila some concrete evidence has been supplied by Yaffe (1956), and our own experiments with chicken embryos have furnished considerable evidence that interference with coenzymes, and especially with codehydrogenases, plays an important role in the origin of phenocopies. This prominent role of the codehydrogenases, in our material at any rate, is of interest because it agrees on the one hand with the preferential vulnerability of certain organs and systems while, on the other hand, the great versatility with which codehydrogenases function in a multiplicity of biochemical reactions helps us to understand dissimilar phenocopic responses which are brought about by a disturbance of related, but not identical, enzymatic functions. As an example of the latter kind we refer to the fact that quite disparate phenocopies (with a 100% incidence) are produced in chicken embryos of the same age and stock by treatment with 6-aminonicotinamide and 3-acetylpyridine (micromelia with abnormal beak and muscular hypoplasia, respectively), but that complete protection against both teratogens is provided by nicotinamide as well as tryptophan (Landauer, 1957b). Other observations might be used to exemplify the same relations, for instance the possibility of differential protection against parts of a phenocopic syndrome, a possibility which is presumably based on intra-embryonic competition for essential metabolites and which has also been encountered as a result of the modification of pleiotropic gene action by the environment (e.g., Nozawa, 1956). Of great interest in this respect is also the prominent role which (in vertebrates) vitamin deficiencies play in the production of phenocopies. In short, the role played by coenzymes permits a reasonable interpretation of the generalized as well as of the specialized aspects of phenocopy production.

## SUMMARY

To sum up: Recognizing that certain facts remain which at present defy interpretation and which call for further analysis, I believe that the evidence which has thus far been brought forward from various sources justifies the following interpretation of the genesis of phenocopies. As mutant genes produce a dislocation in the integrated functions of the normal geno-

type, so do certain external agents, whether physical or chemical, intervene in gene-determined developmental events by preventing them at one point or another from accomplishing their appointed ends, leading thereby to a mutant-like phenotype. The point of interference varies according to the existing genotype, the developmental stage at intervention, kind and quantity of the external agent, and still other factors. But I believe that in all instances the resulting phenocopies are the result of external pressure which has a suppressing, retarding or disorienting effect on one or more gene-controlled components of the normal or mutant genotype in question. It is probable that with sufficient or the proper kind of pressure many gene-controlled events can be made to appear as being the result of sub-threshold alleles, but I do not believe that the existence of such isoalleles as determiners of phenocopies has been proven or that it is likely.

I believe that the phenotypic homologies between mutants and phenocopies, whether they be only superficial or intrinsic, represent two different ways in which weaknesses of ontogenetic integration are revealed. The fact that, in our experience, those mutant phenotypes which exist as several independent gene substitutions are also the ones which can most readily be produced as phenocopies, and that the affected events depend on complex developmental interactions presumably enhances the likelihood of "derailments" to occur.

The deficiencies in developmental integration and stability which we have in mind must, of course, be viewed as a failure of evolution to provide mechanisms which could cope with the genic or environmental sources of dislocation. There is abundant evidence, however, for the widespread occurrence of specific modifiers, as constituents of the residual genotype, which in a similar manner alleviate mutant and phenocopic development. This is important in view of the many facts which suggest that these modifiers are in reality an integral, if varying, part of the normal genic endowment of organisms.

Taking all evidence into account it seems to me that the most exciting aspect of the study of phenocopies is the opportunity it may provide of shedding light, if only indirectly, on the developmental functions of that awesome skeleton in the closets of genetical science–the normal genotype.

## LITERATURE CITED

Bertschmann, M., 1955, Versuche zur phänokopierenden Wirkung von Chemikalien (Nitrogen-Mustard U. A.) bei *Drosophila melanogaster.* Zeits. indukt. Abst. u. Vererb. 87: 229-266,

Bodenstein, D. and A. Abdel-Malek, 1949, The induction of aristapedia by nitrogen mustard in *Drosophila virilis.* Jour. Exp. Zool. 111: 95-115.

Dobzhansky, Th., 1930, Genetical and environmental factors influencing the type of intersexes in *Drosophila melanogaster.* Amer. Nat. 64: 261-271.

Dobzhansky, Th. and C. B. Bridges, 1928, The reproductive system of triploid intersexes in *Drosophila melanogaster.* Amer. Nat. 62: 425-434.

Dunn, L. C. and W. Landauer, 1934, The genetics of the rumpless fowl with evidence of a case of changing dominance. Jour. Genet. 29: 217-243.

1936, Further data on genetic modification of rumplessness in the fowl. Jour. Genet. 33: 401-405.

Glass, B., 1957, In pursuit of a gene. Science 126: 683-689.

Glass, B. and H. L. Plaine, 1952, The role of oxygen concentration in determining the effectiveness of X-rays on the action of a specific gene in *Drosophila melanogaster.* Proc. Nat. Acad. Sci. 38: 697-705.

Gloor, H., 1947, Phänokopieversuche mit Äther an Drosophila. Rev. Suisse Zool. 54: 637-712.

Goldschmidt, R. B., 1935, Gen und Ausseneigenschaft (Untersuchungen an Drosophila. I. II. Zeits. indukt. Abst. u. Vererb. 69: 38-131.

1954, Different philosophies of genetics. Proc. 9th Internat. Congr. Genetics. Caryologia 6, Supplement, Part 1: 81-99.

1957, Problematics of the phenomenon of phenocopy. Jour. Madras Univ. B 27: 17-24.

Goldschmidt, R. B. and L. K. Piternick, 1957a, The genetic background of chemically induced phenocopies in Drosophila. Jour. Exp. Zool. 135: 127-202.

1957b, The genetic background of chemically induced phenocopies in Drosophila. II. Jour. Exp. Zool. 136: 201-228.

Green, M. C., 1957, Modifiers of the pleiotropic effects of the short ear gene in the mouse. Jour. Hered. 48: 205-212.

Hadorn, E., 1955, Letalfaktoren in ihrer Bedeutung für Erbpathologie und Genphysiologie der Entwicklung. Stuttgart (Georg Thieme).

Kaji, S., 1954, Experimental studies on the developmental mechanism of the Bar eye in *Drosophila melanogaster.* I. Effect of ureids and acid amides on the facets of the Bar eye. Annot. Zool. Jap. 27: 194-200.

1955, Experimental studies on the developmental mechanism of the Bar eye in *Drosophila melanogaster.* II. On the chemical structure of the substances having the facet-increasing effect. Annot. Zool. Jap. 28: 152-157.

1956, Experimental studies on the developmental mechanism of the Bar eye in *Drosophila malanogaster.* III. Influence of the N-fatty acid amides on the facet-formation of the Bar fly. Annot. Zool. Jap. 29: 23-27.

Landauer, W., 1946, The results of selection against the expression of the "short upper beak" mutation of fowl. Amer. Nat. 80: 490-494.

1947, Insulin-induced abnormalities of beak, extremities and eyes in chickens. Jour. Exp. Zool. 105: 145-172.

1948, The phenotypic modification of hereditary polydactylism of fowl by selection and by insulin. Genetics 33: 133-157.

1954, On the chemical production of developmental abnormalities and of phenocopies in chicken embryos. Jour. Cell. Comp. Physiol. 43, Suppl. 1: 261-305.

1955, Recessive and sporadic rumplessness of fowl: effects on penetrance and expressivity. Amer. Nat. 89: 35-38.

1956a, Hereditary and induced cross-beak of fowl. Jour. Exp. Zool. 132: 25-38.

1956b, The teratogenic activity of pilocarpine, pilocarpidine and their isomers, with special reference to the importance of steric configuration. Jour. Exp. Zool. 132: 39-50.

1957a, Phenocopies and genotype, with special reference to sporadically-occurring developmental variants. Amer. Nat. 91: 79-90.

1957b, Niacin antagonists and chick development. Jour. Exp. Zool. 136: 509-530.

Nozawa, K., 1956, The effects of the environmental conditions on curled expressivity and the interaction between two mimic genes curled and Curly, in *Drosophila melanogaster.* Jap. Jour. Genet. 31: 321-326.

Ogaki, M., 1957, Mechanism of manifestation in the Bar-eyed mutant of *Drosophila melanogaster.* Proc. Internat. Genet. Symposia, Tokyo, 1956, pp. 153-155.

Rapoport, J. A., 1939, Specific morphoses in Drosophila induced by chemical compounds. Bull. Biol. et Medécine Exp. de l'URSS 7: 414-417.

Sang, J. H. and J. M. McDonald, 1954, Production of phenocopies in Drosophila using salts, particularly sodium metaborate. Jour. Genet. 52: 392-412.

Sturkie, P. D., 1943, Suppression of polydactyly in the domestic fowl by low temperature. Jour. Exp. Zool. 93: 325-346.

Valadares-da-Costa, M. and R. Jacquot, 1952, Effects d'une surcharge en tryptophane sur le développement et la coloration de l'oeil des mutants v et vbw de *Drosophila melanogaster* en fonction du taux protidique de milieu. Compt. Rend. Acad. d. Sci. 234: 1214-1216.

Waddington, C. H., 1957, Research Report 1955-57, Inst. Animal Genetics, Edinburgh University

Wahab, A. A.-E., 1957, The determination of facet number in *Drosophila melanogaster.* I. The effect of ureids. II. The effect of acid amides. Research Report 1955-57, Inst. Animal Genetics, Edinburgh University.

Warren, D. C., 1944, Inheritance of polydactylism in the fowl. Genetics 29: 217-231.

Yaffe, D., 1956, An experiment on the enzymatic basis of the phenocopic effect produced by silver nitrate. Bull. Res. Counc. Israel 5 B: 315-316.

Vol. XCII, No. 865 The American Naturalist July–August, 1958

# RELATIVE FITNESS OF WILD HOUSE MICE HETEROZYGOUS FOR A LETHAL ALLELE

L. C. DUNN, A. B. BEASLEY AND HARRIET TINKER*

Columbia University, New York, New York

Among the factors tending to change the frequencies of genes in populations of animals, the relative fitness or adaptive value of the several genotypes is generally of controlling importance. In an increasing number of cases, some of the variant alleles are found to have low fitness or are lethal when homozygous; yet are maintained in a state of balanced polymorphism by the superiority of the heterozygotes. Many instances of this have been reported in insect populations (review in Sheppard, 1958). The existence of some mechanism involving selective advantage of heterozygotes is to be suspected wherever alleles which are deleterious when homozygous occur at equilibrium levels above those attributable to mutation pressure. This is the case in man with the gene for sickle cell anemia which has a high frequency in some populations in which falciparum malaria is hyperendemic. There is some evidence (Allison, 1954) that heterozygotes have greater resistance to malaria. A similar situation may obtain with the gene for thalassemia.

Other mammalian populations have presented few opportunities for analyzing the causes of polymorphism. Recently, however, it has been found that wild populations of *Mus musculus* in many parts of the United States are polymorphic for variant alleles ($t^w$ alleles) generally lethals, at one locus *T* (Dunn, 1956). Such alleles are not only widespread but occur in some populations in frequencies much higher than could be maintained by mutation (Dunn, 1956).

In a search for causes by which this polymorphism might be maintained, it was found that wild male heterozygotes transmit the mutant $t^w$ alleles to a great majority (about 96 per cent) of their offspring (Dunn 1957a). The advantage thus conferred on the mutant allele is sufficient not only to compensate for the loss of such alleles by lethality but to lead to very high frequencies of them when equilibrium values are computed with assumptions of random mating, complete selection against homozygotes and equal fitness of +/+ and $+/t^w$ (Dunn, 1953; Prout, 1953; Bruck, 1957). In this model, the equilibrium frequencies of $t^w$ alleles in wild populations tend toward 50 per cent (100 per cent heterozygotes) as the ratio of $t^w$ to + gametes approaches its limit of 1.00. With these assumptions, we should expect to find very high frequencies of heterozygotes in any population in which a "high-ratio" allele occurs. In the small samples of mice tested from 14

*Work carried out at Nevis Biological Station, Irvington-on-Hudson, New York, under Contract AT (30–1)1804 with the U. S. Atomic Energy Commission.

wild populations in which $t^w$ alleles were found, the average frequency of heterozygotes was 50 per cent or less.

Since the actual state of polymorphism in nature does not correspond to that predicted from the model, one may suspect that one or more of the assumptions underlying the model are wrong. The first parameter, male segregation ratio, has been determined experimentally for all 16 alleles taken from the wild. It varies for different alleles from .9 to .99 with an average of .96 (Dunn, 1957a), that is, close to its limit of 1.0 as in the model. A second assumption subject to experimental test is the equal fitness of +/+ and $+/t^w$. It would be reasonable to expect that heterozygotes are less fit, since if the "segregation ratio" advantage is sufficient to produce heterozygote frequencies close to 100 per cent (and less than this is found) then other evolutionary forces must oppose the spread of the lethal, and of these the most likely is selection.

A preliminary test of the hypothesis of heterozygote disadvantage (Dunn and Suckling, 1956) failed to support it. In fact there was an indication that male heterozygotes were more fertile than normals. The present paper reports a more extensive repetition of this test and again indicates that males heterozygous for a lethal are superior to their homozygous normal brothers in some of the elements of fitness.

## DESIGN OF THE EXPERIMENT

It is required to test a population in which the two genotypes are present at birth in known proportions, to observe any change in this ratio as the population ages, and to compare the reproductive efficiencies of the two genotypes under standard conditions. Such a test population was produced by mating wild females heterozygous for a lethal allele ($t^{w11}$, Dunn, 1957b) by wild males from the same population (Connecticut 2, Dunn, 1957b), the genotypes of each animal having been established by previous progeny testing. The gametic ratio from $+/t^w$ females has been shown repeatedly to be normal (.5). The 14 $+/t^{w11}$ females used to produce the test population were progeny-tested by Brachy (T/+) mates. The ratio of their + and $t^w$ gametes is given by the ratio of Brachy (T/+) and tailless ($T/t^w$) in the test progenies. This was 56 T/+ : 43 $T/t^w$; the gametic ratio thus does not depart significantly from .5 ($p = 0.23$).

These 14 females ($+/t^w$) produced when mated with +/+ wild males a test population at birth of 138 animals (67 ♂♂ and 71 ♀♀) all of normal phenotype. Since the probability of occurrence of the two genotypes +/+ and $+/t^w$ is equal at birth in this population it is now required to mate these in such a way as to reveal the actual proportions of the two genotypes and to measure their relative reproductive efficiencies. This was done by mating all to Brachy (T/+) animals derived from inbred Brachy stocks and from crosses between these stocks to ensure maximum vigor. All Brachy animals used were previously tested for fertility and only those were used which had given five or more offspring in the first litter. A pool of such animals was established from which replacements were provided for the test matings.

## RESULTS

Conclusive results were obtained only from the males of the test population. In spite of special provisions, such as exercise wheels, to encourage wild females to breed, only 21 out of 71 produced enough offspring for a progeny test. The males were tested individually by mating at 28 days of age with four tested Brachy females. As females became pregnant they were removed to isolation pens in which the litters were born. Each female so removed was immediately replaced by a tested Brachy female from the pool. Litters were scored and killed at birth and the female then returned to a different male. Each male was thus continuously mated to four females, and the Brachy females circulated through the test pens in such a way that each male had access to a random sample of fertile females from the pool.

For each female there was recorded the date of birth of each litter and the number and phenotypes of the offspring at birth. These records were obtained for 53 males, each of which had been mated for 16 weeks (until 140 days of age) and had produced at least 10 offspring each, thus permitting a secure diagnosis of genotype. Of the 14 males which did not complete the test, 5 died before weaning, 2 died between one and two months of age, 3 produced only one litter each, not constituting a progeny test, and 4 produced no litters after 16 weeks of mating and were judged to be sterile.

The 53 tested males divided themselves clearly into two groups on the basis of the progeny tests. One group regularly produced normal, Brachy, and tailless offspring, leading to a diagnosis of $+/t^w$, with $t^w$ gametes in great excess (94 per cent), as in previous tests of $T/t^w$ and $+/t^w$ males. The other group produced only normal and Brachy offspring in about equal frequency. There were 35 $+/t^w$ and 18 +/+ males. This deviation from equality is significant ($p = .03$) suggesting selection against +/+ in the period before the genotypes could be distinguished. The offspring produced by these two genotypes are classified in table 1.

TABLE 1

| | Normal | Brachy | Tailless | Total | No. of litters | Average per litter | Offspring per ♂ | Age at 1st litter | Litters per ♂ |
|---|---|---|---|---|---|---|---|---|---|
| 35 $+/t^w$ ♂♂ | 983 | 47 | 845 | 1875 | 311 | 6.03 | 53.57 | 73.97 | 8.9 |
| 18 +/+ ♂♂ | 539 | 499 | ... | 1038 | 151 | 6.87 | 57.70 | 66.50 | 8.4 |

Apart from the striking discrepancy in the proportion of the two genotypes present at sexual maturity, they are nearly equal in reproductive efficiency. Thus the heterozygotes constituted 66 per cent of the population at maturity, produced 64 per cent of the progeny and 67 per cent of the litters. A slight superiority of the +/+ males is suggested by the data on litter size, number of offspring per male, and age at maturity as judged by first litter.

The latter difference (7.47 ± 11.34) is the most extreme but it is not statistically significant.

## DISCUSSION

The significant difference between the two genotypes, as far as this evidence goes, is their unequal representation in the population at sexual maturity. This points to a selective advantage of heterozygous males in early life. Since only four sterile males were found out of 53 tested, the difference cannot be due to this cause and the previous test (Dunn and Suckling, 1955) which indicated a higher sterility rate for +/+ males is not confirmed. A more probable cause is superior viability of heterozygous males between conception and sexual maturity.

From the data in table 1 it is possible to estimate the proportion of $t^{w11}$ and + alleles transmitted to the next generation by the 53 tested males. The Brachy ($T/+$) and tailless ($T/t^{w11}$) offspring from $+/t^{w11}$ males result from fertilization of $T$ eggs by + and $t^{w11}$ sperm respectively. The proportion of $t^{w11}$ sperm is thus $\frac{845}{845+47} = .947$. The total number of $t^{w11}$ alleles transmitted is thus $.947 \times 1875 = 1775$. The remainder of the offspring from $+/t^{w11}$ males ($1875-1775=100$) can be ascribed to fertilization by + sperm. The 18 +/+ males contributed 1038 + alleles. The frequency of $t^{w11}$ therefore is $\frac{1775}{1775+1038+100}$, that is, 60.9 per cent of the gametes transmitted were $t^{w}$. The proportion expected to be transmitted by a population in which half the animals are heterozygotes $+/t^{w11}$ with normal segregation and without selection is of course 25 per cent. Thus the test population males transmitted nearly two and a half times as many $t^{w}$ alleles as expected. A large part of this excess transmission of $t^{w}$ is due to the segregation ratio advantage (.947) of $t^{w}$ sperm. The part due to selective advantage of $+/t^{w}$ males can be estimated as follows. If +/+ and $+/t^{w}$ are selectively equal each should produce half of the total progeny, in this case $2912/\ 2 = 1456$. Of those produced by $+/t^{w}$ males 94.7 per cent of 1379.3 should be from $t^{w}$ sperm. This is 47.4 per cent of all alleles transmitted. We may say therefore that segregation ratio advantage has increased the transmission of $t^{w11}$ alleles by this test population from an expected 25 per cent to 47.4 per cent. Since the observed proportion of $t^{w}$ alleles found after selection is greater than this (60.9 per cent), an additional increment has been added by selection.

It is of interest to compare the magnitudes of the effects of "segregation ratio" and selection. These two forces are not entirely comparable, since the effects of a given "segregation ratio" (m) or adaptive value (W) on the allele frequency will depend in different ways on the initial allele frequency.

The relative effects of m and W may be estimated as follows. Let p be the frequency of the allele $t^{w}$ in the initial population, and let $p'$ be the frequency of the $t^{w}$ allele at conception of the next generation and $p''$ be

the frequency of the allele at maturity of the next generation. Let x be the ratio of $t^w$ to + alleles; $x = \frac{p}{1-p}$ and $p = \frac{x}{1+x}$; and let the ratios $x'$ and $x''$ be similarly defined. Then the allele frequency in the next generation will be $p' = rp$ where $r = 2m$, i.e., twice the segregation ratio m (in the normal case $m = .5$, $2m = 1$); and the allele ratio in the next generation at sexual maturity will be $x'' = Wx'$, where W (adaptive value) is the effect of selection alone.

For purposes of comparison, the effect of segregation ratio may be estimated by $\frac{x'}{x}$ where $x = \frac{p}{1-p} = \frac{.25}{.75} = .333$; and $x' = \frac{p'}{1-p'} = \frac{.474}{.526} = .900$. $\frac{x'}{x}$ for segregation ratio is thus $\frac{.900}{.333} = 2.73$. The effect of selection may be estimated by $\frac{x''}{x'}$ where $x' = .900$ and $x'' = \frac{p''}{1-p''} = \frac{.609}{.391} = 1.56$ giving $\frac{x''}{x'} = 1.73$. Since both of these quantities multiply x it is reasonable to compare their logs: log 1.73 = .238; log 2.73 = .436. Thus selection contributes $\frac{.238}{.238 + .436} = .35$ or about one third of the total effect while segregation ratio contributes about two thirds.

The above estimates are derived from comparisons between heterozygotes ($+/t^w$) and homozygotes (+/+) when only males are considered. The evident superiority of heterozygous males might be offset by a corresponding inferiority of heterozygous females. Our data are not sufficient to test this possibility. Only 19 test females produced sufficient progeny (5 or more Brachy offspring) to constitute a test of genotype. Of these 19, 9 proved to be $+/t^w$, 10 to be +/+. There is thus no evidence of inequality in the numbers of the two genotypes. The experiment must be repeated with larger numbers.

However, it must be said that a disadvantage in heterozygous females would have to be very great indeed if it were to offset the advantage in the males. This is because the heterozygous males, by virtue of the segregation ratio advantage, play the predominant role in determining the gene frequency in the next generation. Selective superiority of heterozygous males has therefore a much greater effect on gene frequency than an equal advantage of heterozygous females; and a more than equal disadvantage of heterozygous females would be required to counteract it.

The results of the present tests, therefore, while not complete, point to a net reproductive advantage of animals carrying a lethal. There is no evidence of the selective inferiority of heterozygotes which we had been led to expect from the fact that the frequencies of *t*-alleles in wild populations are, as far as our limited data go, below the equilibrium frequencies computed, having regard to the known segregation ratio advantage of $t^w$ alleles from heterozygous males. At present both evolutionary forces—segregation ratio and selection—appear to act in the same direction. Since one of these

alone—segregation ratio—was sufficient to produce frequencies of *t*-alleles higher than those found in nature, it is evident that other forces, at present unknown, must act in the opposite direction to oppose increases in gene frequencies of these alleles. These influences must be sought in the conditions under which the wild populations live, especially the population structure in respect to size of breeding units, intensity of inbreeding, and similar factors. At present we know too little about these to support speculation.

## SUMMARY

An attempt has been made to estimate the relative fitness of wild mice of two genotypes, those with (+/$t^w$) and without (+/+) a lethal allele at one locus (T). Starting with a population of 67 males at birth, in which the probability of each genotype was equal, it was found to consist at 140 days of 35 +/$t^w$ and 18 +/+ animals, the difference being significant at the 0.03 level. The total number of offspring produced by the two genotypes was +/$t^w$ 1875, +/+ 991, a ratio of net reproductive efficiencies of 1.89 to 1. Of the net gain in frequency of the $t^w$ allele between the test population and their progeny, about 63 per cent was due to the segregation ratio advantage of heterozygous males and about 37 per cent to their selective advantage. Data from wild females were insufficient but did not indicate a disadvantage of heterozygous females sufficient to counteract the effect on gene frequency of the advantage enjoyed by heterozygous males.

It is concluded that two evolutionary forces—male segregation ratio advantage and selection—both act to increase the frequency of such lethals in populations. Other forces probably act in the opposite sense to produce the equilibrium values of these alleles. These forces remain to be identified.

## LITERATURE CITED

Allison, A. C., 1954, Protection afforded by sickle-cell trait against subtertian malarial infection. Brit. Med. Jour. 1: 290–292.

Bruck, David, 1957, Male segregation ratio advantage as a factor in maintaining lethal alleles in wild populations of house mice. Proc. Nat. Acad. Sci. 43: 152–158.

Dunn, L. C., 1953, Variations in the segregation ratio as causes of variations in gene frequency. Acta Genet. et Statist. Med. 4: 139–151.

1956, Analysis of a complex gene in the house mouse. Cold Spring Harbor Symposium 21: 187–195.

1957, Evidence of evolutionary forces leading to the spread of lethal genes in wild populations of house mice. Proc. Nat. Acad. Sci. 43: 158–163.

1957, Studies of the genetic variability in populations of wild house mice. II. Analysis of eight additional alleles at locus T. Genetics 42: 299–311.

Dunn, L. C. and Joan A. Suckling, 1955, A preliminary comparison of the fertilities of wild house mice with and without a mutant at locus T. Amer. Nat. 89: 231–233.

Prout, Timothy, 1953, Some effects of variations in the segregation ratio and of selection on the frequency of alleles under random mating. Acta Genet. et Statist. Med. 4: 148–151.

Sheppard, Philip, 1958, Natural Selection and Heredity, London.

Vol. XCII, No. 865 The American Naturalist July–August, 1958

# THE NUMBER AND DISTRIBUTION OF INCOMPATIBILITY FACTORS IN SCHIZOPHYLLUM

JOHN R. RAPER, GLADYS S. KRONGELB, AND MARGERY G. BAXTER

Department of Biology, Harvard University, Cambridge, Mass.

Incompatibility mechanisms, replacing or superposed upon sexual differentiation as the agency of discrimination in mating behavior, have evolved in widely separated groups of plants. They are widely distributed and well known in fungi and in flowering plants and only recently have been discovered in a fern (*Pteridium aquilinum*; Wilke, 1953). Both in the fungi and in the flowering plants, there are numerous specific incompatibility systems characterized by various combinations of two to many alleles at one or two loci. A 2-locus, multiple-allelic system, discovered in 1920 and designated *tetrapolarity* by Kniep (1920), occurs commonly among the higher fungi (Hymenomycetes and Gasteromycetes).

In a typical tetrapolar species, the pattern of mating is determined by incompatibility factors belonging to two different series, the *A* factor series and the *B* factor series. Sexual interaction can occur between individuals only in combinations that are heterozygous for both factors, and at meiosis these assort independently to yield progeny of four mating types in equal frequency:

$$A^1A^2B^1B^2 \longrightarrow A^1B^1,\ A^1B^2,\ A^2B^1,\ A^2B^2.$$

Two additional significant facts about tetrapolar sexuality were revealed in subsequent work by Kniep: (a) a number of alternate states of both the *A* and *B* factors were found in collections from different locations (1922) and (b) the progeny of a single fruit body contained occasional *A* and *B* factors that were different from and thus compatibile with the parental factors (1923). Kniep interpreted the former as due to multiple alleles at each of two incompatibility loci and the latter as mutations at the incompatibility loci.

During the succeeding quarter of a century, both the general pattern of tetrapolarity and the occurrence of multiple alternate states of the two incompatibility factors were abundantly confirmed and extended to a large number of diverse species. Whitehouse (1949), in a careful examination of the data relating to "multiple-allelomorph heterothallism" in Hymenomycetes and Gasteromycetes prior to 1949, concluded that in natural populations of tetrapolar Hymenomycetes, alternate states of both incompatibility factors numbered in the order of 100. Shortly thereafter, Papazian (1951) presented evidence from tetrad analysis that strongly indicated the *A* factor of *Schizophyllum commune* to comprise more than a single locus, with intra-

factor recombination resulting in new factors that were interfertile with both parental *A* factors.

An extended study of the incompatibility mechanism in *S. commune* was initiated in this laboratory in 1955 as a part of a broad examination of the sexuality of this species. Two primary objectives of this study were (a) to determine the magnitude of the two incompatibility series in nature with as great accuracy as practical and (b) to establish the genetic structure of the incompatibility factors. The current paper presents the results of this work that pertain to the first of these objectives: the number and distribution of incompatibility factors.

## SOURCES OF MATERIALS AND TESTING PROCEDURE

The materials for this study originated from widely scattered points all over the world (fig. 1). A sizable collection of strains from Midwest U.S. and New England was already available and had been partially analysed for incompatibility factor content when, in October 1956, a request for additional material was sent to 37 foreign stations. The response was most gratifying: one to several collections of fresh fruit bodies were received from all but a very few of the persons to whom the request was sent (cf. Acknowledgements). Of the total sample of 114 distinct strains, about half came from collections made in the U.S., and about half of these were collected in Massachusetts, within a radius of 10 miles of Lexington, Mass.

The procedure for the identification of incompatibility factors is simple. From a particular fruit body, either collected from nature or originating from a dikaryotic mycelium in the laboratory, a few single spores were isolated. After a few days' incubation, a single monosporous strain was chosen and mated with other members of the sample. A sexual reaction (see below) in a pairing identified the two mates as containing all four of the specific incompatibility factors of the parental, dikaryotic stock. Two strains thus obtained from each of many stocks of divers origins were then mated together in all possible combinations. Matings could be scored after a week's incubation as follows (Papazian, 1950):

(a) dikaryotic mycelium – mated strains contained different *A* and different *B* factors,
(b) common-*A* heterokaryon, "Flat" – mated strains contained the same *A* factor and different *B* factors, and
(c) common-*B* heterokaryon, "Barrage", or no reaction – mated strains contained either different *A* factors and the same *B* factor or the same *A* factor and the same *B* factor.

The two types of matings included in (c) can sometimes be distinguished because of the "barrage" reaction in the different-*A*, common-*B* interaction. The "barrage" reaction has been found to be unreliable in actual practice, and further tests are required whenever unambiguous distinction between the two types of matings included in (c) is necessary.

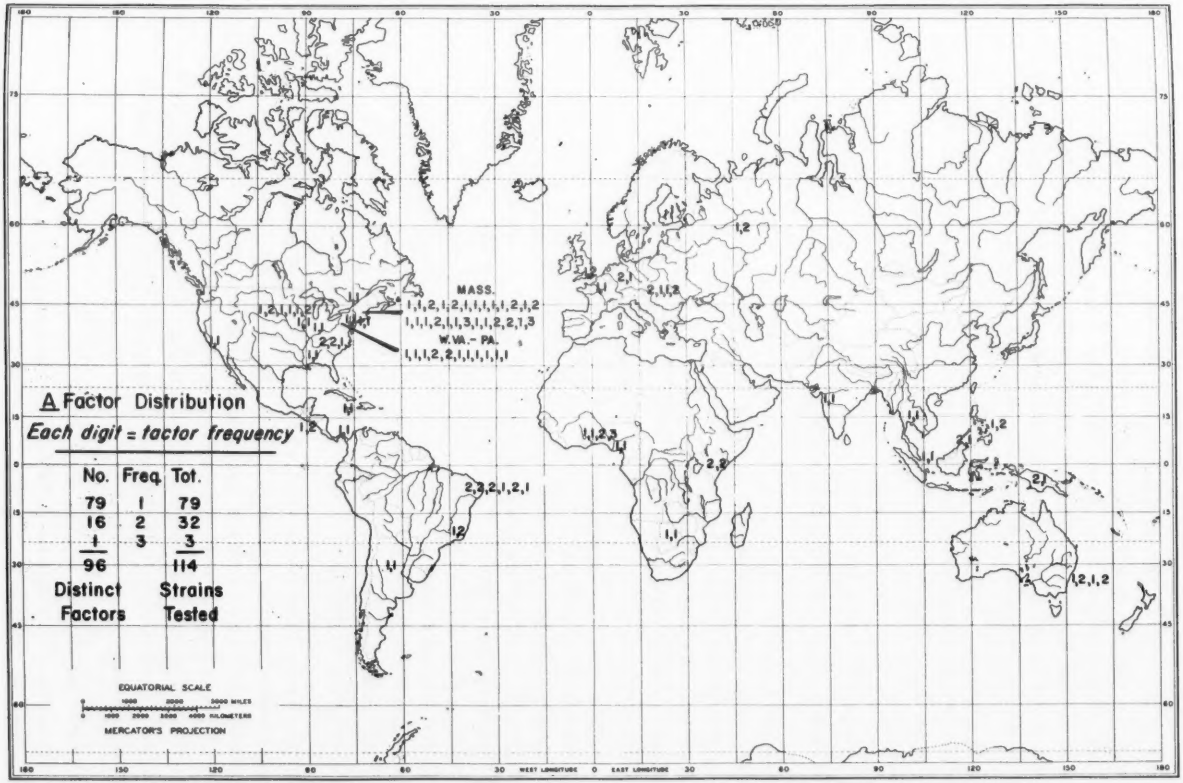


FIGURE 1. The distribution of *A*-factors of *Schizophyllum commune*. Each digit represents the location of an *A*-factor collected from nature, and the value of the digit represents the number of times the specific factor was recovered from the total sample. The summary table at the left gives the frequency distribution for specific *A*-factors.

### THE DISTRIBUTION OF *A* AND *B* INCOMPATIBILITY FACTORS

In the sample of 114 homokaryotic strains, there were 96 distinct, compatibile *A* factors. Of these, a single one was found in strains from three different locations, 16 were each found twice, and the remaining 79 were each recovered only a single time. In figure 1 are plotted the collection sites of all the strains tested; each strain is indicated by a single digit, and the value of the digit represents the number of times that the specific *A* factor was recovered in the total sample. For example, the two *A* factors from Mozambique, Portuguese East Africa, each occurred twice in the sample: one was identical with a factor from Wisconsin, the other, with one from Australia. The matched localities of replicated *A* factors were as follows:

Collected three times:
Massachusetts – Massachusetts – Gold Coast, Africa

Collected twice:
Wisconsin – Massachusetts
Massachusetts – Germany
New Guinea – Czechoslovokia
Massachusetts – Pennsylvania
Massachusetts – Pennsylvania
Massachusetts – England
Massachusetts – Brazil
Australia – Mozambique, Africa
Massachusetts – North Carolina
Wisconsin – Mozambique
Brazil – Brazil
Brazil – North Carolina
Brazil – North Borneo
Philippines – Czechoslovokia
Costa Rica – Australia

With one conspicuous difference, the occurrence of *B* factors in the natural population generally resembled that of the *A* factors. The frequency of replication of *B* factors was about five times greater than for the *A* factor (fig. 2). There were only 56 distinct *B* factors in the sample, and of these, one occurred six times, one five times, 6 four times, 9 three times, 13 twice, and 26 only once. The specific origins of all *B* factor replications were as follows:

Collected six times:
Wisconsin – W. Virginia – New Guinea – Massachusetts – Germany – England

Collected five times:
Connecticut – Massachusetts – Massachusetts – Wisconsin – France

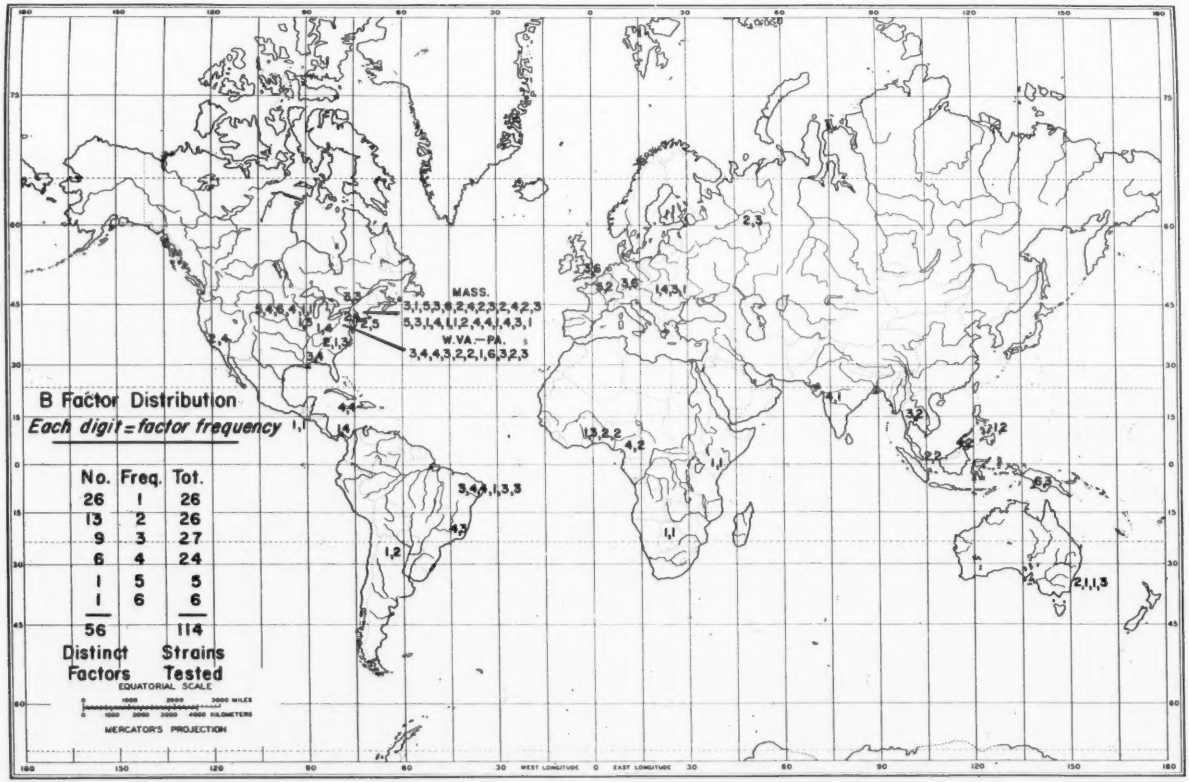


FIGURE 2. The distribution of *B*-factors of *Schizophyllum commune*. Each digit represents the location of a *B*-factor collected from nature, and the value of the digit represents the number of times the specific factor was recovered from the total sample. The summary table at the left gives the frequency distribution for specific *B*-factors.

Collected four times:

New York – Massachusetts – Czechoslovokia – Jamaica
Wisconsin – Massachusetts – Indiana – Brazil
California – Wisconsin – Brazil – Nigeria
Pennsylvania – Massachusetts – Brazil – North Borneo
Massachusetts – Pennsylvania – Massachusetts – Jamaica
Massachusetts – Alabama – Panama – India

Collected three times:

Illinois – Massachusetts – Canada
Massachusetts – England – Russia
Pennsylvania – Massachusetts – Tennesse
W. Virginia – W. Virginia – New Guinea
Massachusetts – Canada – Australia
Alabama – Brazil – Brazil
Germany – Massachusetts – Brazil
Thailand – Gold Coast – Czechoslovokia
Massachusetts – Pennsylvania – Brazil

Collected twice:

New York – Massachusetts
California – North Carolina
Connecticut – Malaya
Massachusetts – Pennsylvania
W. Virginia – Nigeria
Massachusetts – France
Massachusetts – Gold Coast
Massachusetts – Russia
Massachusetts – Thailand
Australia – Argentine
Pennsylvania – Malaya
Pennsylvania – Gold Coast
North Borneo – Phillipines

A casual inspection of the data on replications of *A* and *B* factors reveals no obvious departure from random distribution of *A* or *B* factors in respect either to geographical location or to climatic conditions. Two simple tests have been applied to test an hypothesis of randomness.

A. A test for difference in proportion of factor replications, that is, factor identities, in intra- and inter-regional matings revealed no difference at the 5 per cent level of significance. The sample was divided into five regional samples: United States-Southern Canada, Central-South America, Europe, Africa, Southeastern Asia-Australia. The proportionality of factor replications to factor pairs among all crosses between strains from the same geographical region was then compared with the proportionality of replications in crosses between strains originating from different regions

TABLE 1

INTRA- AND INTER-REGIONAL DISTRIBUTION OF FACTOR REPLICATION

| | Sample | Region |
|---|---|---|
| A | 60 | United States—Southern Canada |
| B | 16 | Central—South America |
| C | 12 | Europe |
| D | 10 | Africa |
| E | 16 | Southeastern Asia—Australia |
| Total | 114 | |

Intra-Regional

| | No. Crosses | *A* Repl. | *B* Repl. |
|---|---|---|---|
| A | 1770 | 5 | 31 |
| B | 120 | 1 | 1 |
| C | 66 | 0 | 2 |
| D | 45 | 0 | 0 |
| E | 120 | 0 | 2 |
| Total | 2121 | 6 | 36 |
| Proportion | | | |
| | $p_1(A)$ | 6/2121 | |
| | $p_1(B)$ | | 36/2121 |

Inter-Regional

| | No. Crosses | *A* Repl. | *B* Repl. |
|---|---|---|---|
| AB | 960 | 2 | 19 |
| AC | 720 | 2 | 17 |
| AD | 600 | 3 | 5 |
| AE | 960 | 0 | 13 |
| BC | 192 | 0 | 2 |
| BD | 160 | 0 | 1 |
| BE | 256 | 2 | 3 |
| CD | 120 | 1 | 2 |
| CE | 192 | 2 | 2 |
| DE | 160 | 1 | 0 |
| Total | 4320 | 13 | 64 |
| Proportion | | | |
| | $p_2(A)$ | 13/4320 | |
| | $p_2(B)$ | | 64/4320 |

*A* factor:

$p_1 = p_2$, 5% level of significance

*B* factor:

$p_1 = p_2$, 5% level of significance

(table 1). The results of this test provide no evidence of non-random distribution of either *A* or *B* factors.

B. A comparison of frequency of replication of factors in samples of decreasing geographical dimensions gave results that were in agreement with those of (A) above. The same frequency of *A* factor replication – to the nearest whole number – occurred in the total sample, the United States sample, and in the Massachusetts sample. The frequency of *B* factor replication also showed no significant deviation from a constant value in the same three samples. Only two identical pairs were found among the 26 strains collected in Massachusetts; this figure, however, does not differ significantly from the expected value of five ($P = .13$).

The same frequency of replication of both *A* and *B* factors established here had previously been found by Roshal (1951) in a sample – comparable in size to the Massachusetts sample of the present study – taken from a 75-acre tract of forest at Lake Geneva, Wisconsin. The results of the

TABLE 2
FACTOR REPLICATION *VS.* SAMPLING AREA

| | Strains | Crosses | Common *A* Factors | | Common *B* Factors | |
|---|---|---|---|---|---|---|
| | | | No. | Freq. | No. | Freq. |
| Total Sample | 114 | 6441 | 19 | 0.0029 | 100 | 0.0155 |
| United States | 60 | 1770 | 5 | 0.0028 | 31 | 0.0175 |
| Massachusetts (10 mile radius) | 26 | 325 | 1 | 0.0030 | 2 | 0.0061 |
| Lake Geneva, Wis. (0.12 mile radius, Roshal, 1950) | 24 | 276 | 1 | 0.0036 | 5 | 0.0181 |

comparison of these various samples (table 2) again provide no evidence of non-random distribution of either *A* or *B* factors.

One aspect of the work on the genetic structure of the incompatibility factors is germane to the present consideration. The *A* factor is now known (Raper and Baxter, unpublished) to comprise two distinct sub-units – two loci with multiple alleles or two pseudoallelic sites – between which crossing over yields new specific, recombinant *A* factors. In crosses between a single *A* factor from Massachusetts and 11 other *A* factors from widely scattered locations, 15 distinct new *A* factors were recovered as follows: two of the crosses gave no recombination, and each of three classes of recombinants were common to two different crosses. When these 15 recombinant *A* factors were tested with all 96 original *A* factors, 5 were found to be common to the two groups. The geographical origins of the crossed factors and of the duplicated original factors were as follows:

(Massachusetts × Panama) – Wisconsin
(Massachusetts × Illinois) – North Carolina
(Massachusetts × Canada) – Panama
(Massachusetts × Massachusetts) – Pennsylvania, Massachusetts
– Brazil, Massachusetts.

A comparable relationship has now been demonstrated for recombination within the *B* factor, which appears from preliminary tests to be similarly constituted of two sub-units (R. B. Middleton, personal communication). Four recombinant *B* factors were tested against the 56 original factors. Of the four, three were identical with original factors as follows:

(Alabama × Massachusetts-1) ⟩ Massachusetts, Pennsylvania, Brazil
(Alabama × Massachusetts-2) ⟩ Massachusetts, France
(Illinois × Illinois) – California, North Carolina.

### THE FREQUENCY DISTRIBUTION OF SPECIFIC *A* AND *B* FACTORS

The data relating to the occurrence of specific *A* and *B* factors in the sample (tables inset in figs. 1 and 2) have been examined by W. G. Cochran, Professor of Statistics, Harvard University, for evidence of numerical equality or inequality of specific factors in the natural population. An examination of the data revealed no significant departure from numerical equality either in the *A* series or in the *B* series of factors (Cochran, personal communication).

### THE NUMBER OF INCOMPATIBILITY FACTORS

Approximate values for the number of specific *A* and *B* factors in the natural population can be calculated from the frequency of factor replication in the total sample. In the analysis of a comparable problem, the distribution of lethal mutations in Drosophila, Wright (1941) has shown the probability of finding identical members of a series in random pairing to be equal to the reciprocal of the number of alternate members of the series, n, plus a correction for variance,

$$p = 1/n + n\sigma_p 2.$$

Since in the present case any deviation from equal frequency of specific *A* and *B* factors cannot be detected, a first approximation must assume all factors of each series to occur in equal frequency and to be randomly distributed in the population. The probability of finding identical factors in a single trial then becomes 1/n for each series of factors, or $1/n_A$ and $1/n_B$ for the *A* and *B* series, respectively. The error of estimation in this procedure is high, but it can be calculated (Stevens, 1941; Bateman, 1947). The values for the *A* and *B* series would be:

$$A \text{ series: } 1/n_A = \frac{19}{6441} \text{ with 5\% limits of } \frac{11.22}{6441} \text{ and } \frac{29.62}{6441}$$

$$n_A = 339 \text{ with 5\% limits of } 562 \text{ and } 217$$

$$B \text{ series: } 1/n_B = \frac{100}{6441} \text{ with 5\% limits of } \frac{81.52}{6441} \text{ and } \frac{121.56}{6441}$$

$$n_B = 64 \text{ with 5\% limits of } 79 \text{ and } 53.$$

Fisher (1947) objected to the application of an upper limit in a comparable situation, an estimation of the number of *S* alleles in two varieties of clover (Bateman, 1947), on the grounds that the observed replications could occur in an inifinite series. Two conditions were imposed in the Fisher model: the frequencies of the different alleles were in geometric progression and the commonest alleles approached a frequency of 1 per cent. The objection appears to apply to the admissibility of the basic assumption of equal frequency rather than to a value for the upper limit calculated on this assumption.

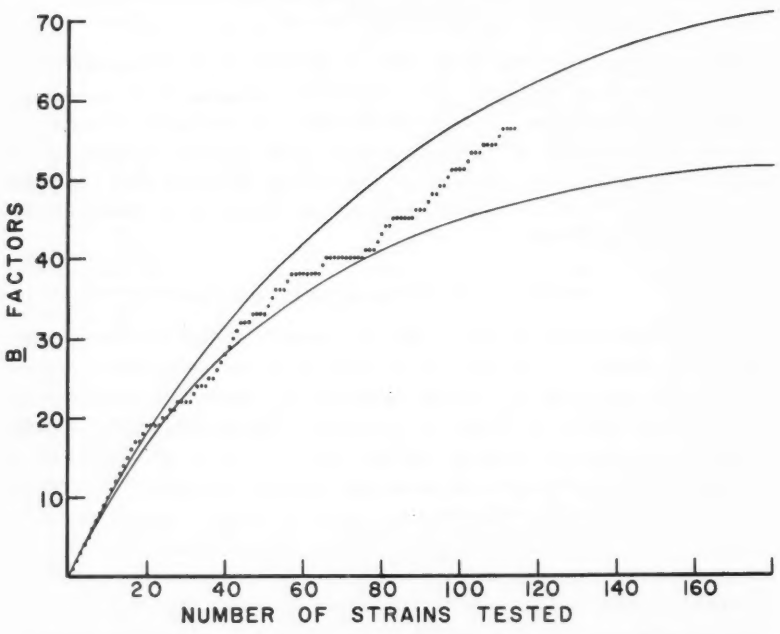


FIGURE 3. The accumulation of specific *B*-factors of *Schizophyllum commune* in a sample of 114 strains plotted in the order of their acquisition (dotted curve). The solid curves represent the expectations of the 5% confidence limits (79 and 53) for the estimated value of 64 distinct *B*-factors in the natural population.

The estimates for $n_B$ appear as entirely reasonable when the pattern of *B* factor replication in the sample is compared with the calculated patterns for $n_B = 79$ and $n_B = 53$. In figure 3 are plotted the accumulation of new specific *B* factors in the sample and the theoretical curves for the 5 per cent limits of $n_B$ as given by the function:

$$B = n_B[1 - e^{-1/n_B \cdot N}],$$

where B is the number of different factors occurring in the sample N. Throughout the range tested in the present sample, the infinite series required by the Fisher model would probably also provide a fair fit. The distinction between a finite series with a central value of $n_B = 64$ and an indefinitely extended series is an experimental possibility. Doubling the size of the present sample should give a significant answer to the uncertainty of the nature of the *B* factor series, but the additional 19,000 matings that would be required to double the sample serve as a cogent deterrant.

The value of the multiple-factor incompatibility mechanism in promoting outbreeding in the groups of plants in which it occurs, the higher fungi and

the flowering plants, has been recognized by Mather (1942), Whitehouse (1949), Papazian (1951), etc. In a tetrapolar form such as Schizophyllum, inbreeding is possible at a level of 25 per cent, but spore dispersal by wind is remarkably efficient, and the upper limit of inbreeding is probably seldom if ever approached. On the other hand, the limits of outbreeding effectiveness can be quite accurately stated. On the twin assumptions of random distribution and equal frequencies of the factors of the *A* and of the *B* series, both of which assumptions are consistent with the data given above, the number of distinct mating types is given by the product of the specific *A* and *B* factors in the population. The estimates of 339 *A* factors and 64 *B* factors would thus provide 21,696 specific mating types, between which pairing can occur in 235,697,360 different combination. Of these possible combinations, the fraction that will oppose different *A* and different *B* factors, that is, will be sexually compatible, is given by:

$$1 - \left(\frac{1}{n_A} + \frac{1}{n_B}\right) = 1 - \left(\frac{1}{339} + \frac{1}{64}\right) = .9815.$$

The total number of sexually fertile combinations is accordingly 231,336,959. A slightly higher efficiency (.9902) would have been achieved with the same total number of specific factors had they been equally divided between the two series.

## SUMMARY

A world-wide sample of 114 homokaryotic strains of the tetrapolar mushroom, *Schizophyllum commune*, was analysed to determine the extent of the "multiple-allelic" series of *A* and *B* incompatibility factors. The sample contained 96 distinct and interfertile *A* factors and 56 different *B* factors. No evidence was found for non-random geographical distribution or for any departure from numerical equality of specific factors either in the *A* series or in the *B* series. Assuming random distribution and equal frequency of factors of both series, these data indicate

339 with 5 per cent limits of 562 and 216 *A* factors and
64 with 5 per cent limits of 79 and 53 *B* factors

in the natural population. Since sexual fertility between strains depends upon heterozygosity of both *A* and *B* factors, the outcrossing efficiency in the natural population is 98.15 per cent.

## ACKNOWLEDGEMENTS

This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (C-2221) and the National Science Foundation (G-3334).

The scope and significance of this work depends in very large part upon the wealth of material that has been kindly provided from stations scat-

tered throughout the world. The authors are pleased to acknowledge their endebtedness to:

A. C. Bastita, Universidade do Recife, Recife, Brazil; K. Cejp, Charles University, Praha, Czechoslovokia; G. Cooley, Cambridge, Mass.; R. Emerson, University of California, Berleley, Calif.; C. L. Fergus, Pennsylvania State College, State College, Pa.; W. P. U. Findlay, Forest Products Research Laboratory, Princess Risborough, Bucks, England; M. A. Hewlett, University College of the Gold Coast, Achimota, Gold Coast; C. T. Ingold, Birkbeck College, University of London, London, England; M. Jacobs, Herbarium Bogoriense, Bogor, Indonesia; M. W. Jennison, University of Syracuse, Syracuse, N. Y.; S. Krongelb, Massachusetts Institute of Technology, Cambridge, Mass.; V. G. Lilly, University of West Virginia, Morgantown, W. Va.; Frances F. Lombard, Forest Service, USDA, Beltsville, Md.; C. J. Magee, Department of Agriculture, Sydney, New South Wales, Australia; P. G. Miles, University of Buffalo, Buffalo, N. Y.; A. Naylor, Department of Agriculture, Jamaica, B. W. I.; D. I. Nicholson, Forest Department, Sandakan, North Borneo; Mildred K. Nobles, Department of Agriculture, Ottawa, Canada; M. M. Payak, Maharashtra Assoc. for the Cultivation of Science, Poona, India; A. Quintanilha, Centro de Investigacao Cientifica, Algodoeira, Mozambique, Portuguese East Africa; E. Quisumbing, National Museum, Manila, The Philippines; J. Y. Roshal, University of Chicago, Chicago, Ill.; Eva Sansome, University College, Ibadan, Nigeria; J. Sinclair, Botanic Gardens, Singapore, Malaya; R. Singer, Fundacion Miguel Lillo, Tucuman, Argentine; P. J. Snider, Harvard University, Cambridge, Mass.; T. Smitinand, Royal Forest Department, Bangkok, Thailand; B. P. Vassilkow, Academy of Science of the USSR, Leningrad, USSR; A. P. Viegas, Instituto Agronomico do Estado de S. Paulo, Campinas, Brazil; G. C. A. van der Westhuzen, National Herbarium, Pretoria, South Africa; Centraalbureau voor Schimmelcultures, Baarn, Holland; American Type Culture Collection, Washington, D. C.; Mycotheque du Laboratoire de Cryptogamie, Paris, France,

## LITERATURE CITED

Bateman, A. J., 1947, Number of *S*-alleles in a population. Nature 160: 337.

Dobzhansky, Th. and Sewell Wright, 1941, Genetics of natural populations. V. Relations between mutation rates and accumulation of lethals in populations of *Drosophila pseudoobscura*. Genetics 26: 23-52.

Fisher, R. A., 1947, Number of self-sterility alleles. Nature 160: 797.

Kniep, H., 1920, Über morphologische und physiologische Geschlechtsdifferzierung. Verh. phys.-med. Ges. Wurzb. 46: 1-18.

1922, Über Geschlechtsbestimmung und Redktionsteilung. Verh. phys.-med. Ges. Wurzb. 47: 1-28.

1923, Über erbliche Änderungen von Geschlechtsfaktoren bei Pilzen. Z. Indukt. Abstamm.-u.-Vererblehre 31: 170-183.

Mather, K., 1942, Heterothally as an outbreeding mechanism in fungi. Nature 149: 54.

Papazian, H. P., 1950, Physiology of the incompatibility factors in *Schizophyllum commune*. Bot. Gaz. 112: 143-163.

1951, The incompatibility factors and a related gene in *Schizophyllum commune*. Genetics 36: 441-459.

Roshal, J. Y., 1950, Incompatibility factors in a population of *Schizophyllum commune*. Thesis, University of Chicago.

Stevens, W. L., 1941, Accuracy of mutation rates. Jour. Genetics 43: 301-309.

Whitehouse, H. L. K., 1949, Multiple allelomorph heterothallism in the fungi. New Phytol. 48: 212-244.

Wilke, D., 1953, Incompatibility in *Pteridium aquilinum* (Bracken). Heredity 7: 150.

Vol. XCII, No. 865 The American Naturalist July–August, 1958

# ON THE EVOLUTION OF HETEROTHALLISM IN FUNGI*

LINDSAY S. OLIVE

Department of Botany, Columbia University, New York City

Since Blakeslee's discovery (1904) of homothallism and heterothallism in the mucors, a large amount of literature has accumulated, most of which has been more concerned with determining whether the species is homothallic or heterothallic rather than attempting to explain how these types of life cycles have evolved. However, several recent papers (Whitehouse, 1949, 1949 a; Raper, 1953; Burnett, 1956) have given consideration to the latter subject. The concepts of these authors and of Papazian (1951), concerning the possible compound nature of the compatibility locus in heterothallic fungi is probably the most satisfactory approach to this problem. The present discussion, which accepts this view of the compatibility locus, is based primarily on recent discoveries of genetic behavior in microorganisms as well as higher organisms, which might explain the origin of heterothallic fungi (as well as other heterothallic lower organisms) from homothallic forms. Unfortunately, clear-cut proof of the hypotheses presented here is not available, for a number of reasons, which will be given later. One of the main purposes of this paper is to alert the investigator to some of the kinds of corroborating evidence that might be expected, with the hope that more information may be brought to bear on this problem in the near future.

It is readily apparent that a system permitting karyogamy between any two haploid nuclei in a thallus (homothallism) is less modified than one involving two types of thalli, the nuclei of which are self-incompatible but cross-compatible (heterothallism). Two-locus or tetrapolar heterothallism, characteristic of most higher basidiomycetes is still more advanced. On the other hand, there is some evidence from the distribution of homothallism and heterothallism in the fungi, particularly in the hymenomycetes, that some homothallic forms may have arisen from heterothallic ones (Whitehouse, 1949 a; Raper, 1953). It is therefore likely that the evolution of these two types of life cycles has proceeded in both directions. This would not alter the assumption that in the early evolution of the fungi homothallism preceded heterothallism.

Although the great majority of fungi have not yet been classified according to their type of life cycle, a cursory examination of the data available shows that both homothallism and heterothallism occur in all classes of fungi. In phycomycetes and ascomycetes, homothallic species are more

*Supported in part by National Science Foundation Grant, NSF G-2808.

common, while in basidiomycetes the great majority of investigated species are heterothallic. There are entire orders of fungi in which heterothallism has not been reported. Certain genera are known (for example, Neurospora) in which all described species are heterothallic (including the pseudohomothallic species), whereas, all investigated species of other genera (for example, Chaetomium) are homothallic. Also of primary interest is the fact that homothallism and heterothallism are distributed throughout the fungi in such a way as to indicate that the latter system has arisen repeatedly in the evolution of the fungi, and likewise, may have reverted to homothallism on various occasions. It further seems likely that these evolutionary processes are continuing at the present time and that, in view of the fact that they probably occur repeatedly, there may be some common genetic mechanism making this possible, which might even be observable in the laboratory.

Before continuing with this discussion, it will be helpful at this point to review the major types of life cycles found in the fungi. The recent system proposed by Burnett (1956) recommending a new set of terms to describe the mating systems of fungi is worthy of note, but for purposes of simplicity is not adopted here.

## MAJOR TYPES OF HOMOTHALLIC AND HETEROTHALLIC BEHAVIOR IN FUNGI

After the discovery of homothallism and heterothallism in all major classes of fungi, it became generally accepted that all sexually reproducing species would fall into one of these two types of life cycles. As early as 1912, however, Edgerton found that the pyrenomycete, *Glomerella cingulata* did not fit either pattern satisfactorily. Since that time, quite a number of species have been found to fall somewhere in between homothallism and heterothallism. Therefore, three major groups of life cycles are described below, with the understanding, however, that these may be further variously subdivided into more concise groups.

*Homothallism.* Although homothallism is commonly defined as the ability of an organism to complete its life cycle from a single haploid meiospore, it has been repeatedly shown in recent years that different genotypes—pathogenicity variants, growth mutants, spore color mutants (figure 1)—of a homothallic species may be readily crossed (Frandsen, 1946; Pontecorvo, *et al.*, 1953; Olive, 1956). Hemmons, *et al*,, (1952) found that certain self-fertile isolates of *Aspergillus nidulans* may be crossed, with the resultant production of considerably more crossed than parental perithecia, a phenomenon which they refer to as "relative heterothallism," a term of dubious value in the absence of more detailed information on the nature of the genetic factors involved. In crosses involving fully self-fertile participants of *Sordaria fimicola,* we have never observed the number of crossed perithecia to exceed 50 per cent of the total at the line of crossing. On the contrary, we have found that different isolates often fail to cross altogether while others cross poorly. Only cultures of the same isolate ever give a fair degree of crossing (Olive, 1956).

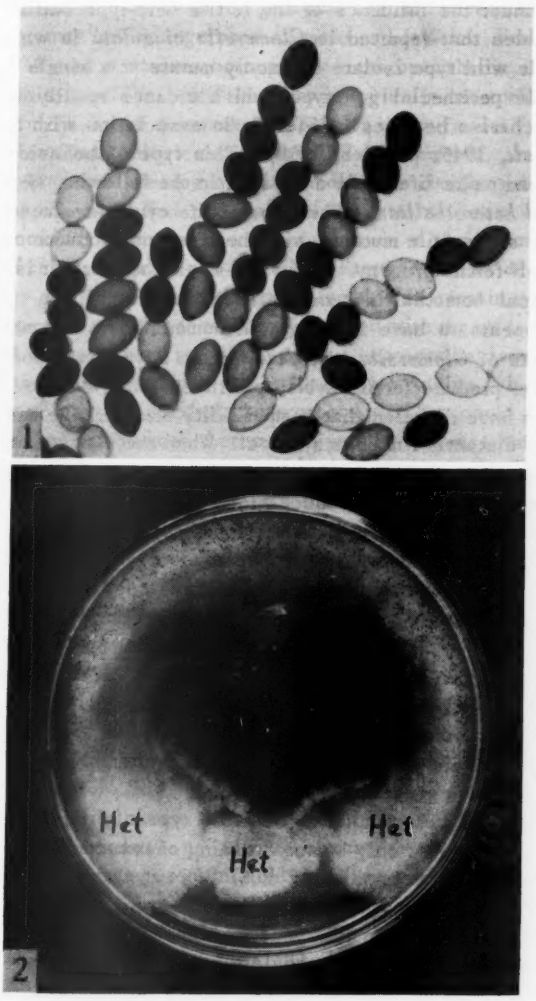


FIGURE 1. Hybrid asci of *Sordaria fimicola* from cross of gray-spored mutant (g) with wild-type (g+). × 620.

FIGURE 2. Fertile heterokaryons (*Het*) arising from anastomosing self-sterile cultures (*A* and *B*) of *Sordaria fimicola.*

Homothallic species may yield either spontaneously or under mutagenic treatment, self-sterile mutants, some of which readily form heterokaryons and crossed perithecia when paired with wild type. My own recent investigations (1956) of such crosses in *S. fimicola* have included a study of spontaneous mutants that are self-sterile when grown alone, but which cross readily with the wild type and even produce some perithecia with homozygous

mutant asci under the influence of the fertile wild-type culture. This behavior resembles that reported in *Glomerella cingulata* in which nuclei of the self-fertile wild type isolate commonly mutate at a single locus to produce a sterile perithecial genotype, which crosses readily with the wild type and which also becomes self-fertile in association with the wild type (Chilton, *et al.*, 1945; Wheeler, 1950). This type of behavior intergrades with the second major life cycle discussed in the following section.

*Unbalanced heterothallism.* This type of life cycle involves the crossing of non-allelic self-sterile mutants, with the resultant production of both self-sterile and self-fertile progeny. In several ways, therefore, it is intermediate between typical homothallism and balanced heterothallism. This type of life cycle appears to have its origin in homothallism. Some of the self-sterile mutants of *Glomerella cingulata*, which developed spontaneously in the laboratory, produce fertile hybrid perithecia when crossed. Studies of these crosses have revealed that compatibility among these mutants is controlled at two distantly linked, major loci. When the almost completely self-sterile, scattered perithecial culture is crossed with the clumped conidial culture, which fails even to produce protoperithecia by itself, hybrid perithecia are produced abundantly, and the ascospore progeny give rise not only to the parental genotypes but also to double mutant and self-fertile recombinants. Essentially similar results have been obtained with irradiation-induced self-sterile mutants of homothallic Sordaria by Greis (1942), Esser and Straub (1956), and Carr and Olive (in press). The term *unbalanced heterothallism* is hereby proposed to identify heterothallic behavior in which self-fertile recombinants are obtained in addition to self-fertile progeny in crosses of non-allelic self-sterile mutants. Variations of this type of behavior are probably more common among the fungi than is generally realized at present.

*True (Balanced) Heterothallism.* In this type of life cycle, the sexual phase can be completed only by the crossing of two compatible self-sterile thalli, and self-fertile recombinants do not appear among the progeny. This is because compatibility is controlled by self-sterility factors at the same locus. *Neurospora sitophila* is a good example. Although each mating type may produce macroconidia, microconidia, and protoperithecia, mature perithecia with asci do not develop unless the two mating types are crossed. This simple, one-locus heterothallism, although found in all major classes of fungi, is probably most common among the ascomycetes. However, in the basidiomycetes, with the exclusion of the rusts and possibly the smut fungi, a somewhat different system is found to be in control of compatibility in heterothallic species. Here we find species with compatibility controlled at one or two loci with multiple allelomorphic factors involved at each locus. This subject will be dealt with in greater detail in a later section.

## PHYSIOLOGICAL VERSUS MORPHOLOGICAL HETEROTHALLISM

Whitehouse (1949 a) has attempted to define two types of heterothallism on the basis of whether the compatible strains of a species are more or less

similar (and hermaphroditic when recognizable sexual structures are present), or whether they may be distinguished as male and female. *Neurospora sitophila*, whose *A* and *a* strains both produce protoperithecia, microconidia and macroconidia, would fall into the "physiologically heterothallic" group, as would the majority of heterothallic fungi. Experimentally proven examples of strict "morphological heterothallism," however, are difficult to find. The ambisexual species of water molds are hardly good examples, since most of the so-called male and female strains show considerable variability in their maleness and femaleness in different matings. For example, in *Achlya ambisexualis* and A. *bisexualis*, some of the females react as males and some of the males as females in certain combinations. Before such species can be adequately discussed with regard to their sexual nature, it will be necessary to obtain careful segregation data from hybrid zygotes, and such data are not yet available for Achlya. It is not even known that these species are truly heterothallic. They could as easily be representatives of unbalanced heterothallism, in which event fertile recombinants of crosses might well prove to be recognizable as presently known homothallic species. As the situation now exists, all recent efforts by ourselves and others to germinate zygotes from these crosses have failed. The zygotes are non-viable. It is therefore possible that the sexual strains now available do not even belong to the same species. The analyses by Couch (1926) of hybrid zygotes from crosses of self-sterile isolates of *Dictyuchus monosporus* indicate that the sexual strains in this species are determined by one-locus heterothallism modified by at least one, and possibly two, additional loci affecting the development of sex organs. Couch did not obtain self-fertile recombinants from the crosses. This would be similar to the situation described in *Hypomyces solani* f. *cucurbitae*, which is discussed in the following paragraph.

Investigators have at times attempted to identify as males or females certain isolates from nature or various mutant cultures obtained in the laboratory. Hansen and Snyder (1946), in their study of the normally heterothallic fungus, *Hypomyces solani* f. *cucurbitae*, found that both *A* and *a* strains are hermaphroditic, self-sterile, and cross-fertile, and that each produces protoperithecia, microconidia and macroconidia when grown alone. They also obtained mutants of these strains, which they call "male" and "female" on the basis that the "male" lacks ascogonia and can fertilize the female with its conidia, but the "female", which produces ascogonia, as well as conidia that are unable to function in plasmogamy, cannot reciprocate. These two genotypes are determined by two additional non-allelic loci that are linked at some distance apart on the same chromosome. Crosses between "males" and "females" occur only when the cross is heterozygous for the *A-a* locus. The progeny contain, in addition to the parental genotypes, recombinant hermaphrodites and double mutant neuters. It is fairly obvious that the "male" and "female" loci are no more than mutant loci blocking the development of protoperithecia or affecting the ability of the conidia to function in plasmogamy. I am in agreement with

Whitehouse (1949 a) that the situation in Hypomyces does not represent a true "haplo-dioecism."

Whitehouse cites as examples of morphologically heterothallic fungi, those dimorphic species of minute insect-parasitizing fungi, the Laboulbeniales, which discharge their ascospores in pairs, one spore developing into a small male thallus alongside a somewhat larger female thallus derived from the other spore. Unfortunately, there is no conclusive evidence that this differentiation is determined by unlike genetic factors. There is the possibility that this dimorphism is no more than a physiological difference and that the thallus which develops more slowly becomes the male, the more vigorous one, the female. If this were true, this system would be an example of "phenotypic heterothallism" somewhat like that reported in *Blastocladiella variabilis* by Emerson (1950). On the other hand, if experimental studies can demonstrate that the so-called male cells are functional as such in these species and that maleness and femaleness are controlled by genetic differences, this would be the most remarkable example of morphological heterothallism known in the fungi.

Recent studies (Spiltoir and Olive, 1955; Spiltoir, 1955) of *Ascosphaera apis* have demonstrated a heterothallic condition that involves distinct morphological differences between the two strains, though not so distinctive as had been reported by earlier investigators. The two mating types have been designated by these investigators as male and female – a terminology that we have also adopted with some reservations. The two mycelia grown separately are entirely vegetative, produce no sex organs, and are fairly similar in appearance. In crosses, the female produces ascogonia, each with a trichogyne that fuses with a vegetative hypha of the male. There are no male organs at all, despite previous reports, although a receptive papillum may appear at the point of plasmogamy on the male hypha. Nuclei pass from the male hypha into the female organ. Ascospores give rise only to male or female mycelia in a 1 : 1 ratio, thus indicating a one-locus control of sexuality. To my knowledge, this is the only proven example of genetically controlled morphological heterothallism known in the fungi, in which no intersexes or self-fertile progeny are produced.

## A GENETIC BASIS FOR THE EVOLUTION OF HETEROTHALLISM

*Compound-locus hypothesis.* As Wheeler (1954) has very logically pointed out, any change in the sexual potency of a homothallic organism will of necessity be in the direction of self-infertility." Furthermore, it is clear from the investigations of Glomerella by Wheeler and co-workers, as well as from the studies of *Sordaria fimicola* by Greis (1942) and ourselves (Olive, 1956; Carr and Olive, in press), that conditions ranging from reduced fertility to complete self-sterility may be brought about by spontaneous or induced mutation at any one of a number of different loci. Wheeler has reported in Glomerella, and we have recently found in Sordaria, that the various mutant loci act as partial or complete genetic blocks inter-

fering with the completion of the sexual process. The stages blocked may be: (1) perithecial initiation, (2) plasmogamy (or hyphal anastomosis in Sordaria), (3) karyogamy, and (4) meiosis, to which might also be added (5) ascospore or germ tube abortion. Wheeler found that most of the mutant loci studied blocked plasmogamy. It is noteworthy that almost any mutant locus, including several responsible for deficiencies in growth requirements (argenine, biotin, thiamin) reduces or prevents self-fertility in Glomerella.

As was mentioned previously, crosses may frequently be made between two non-allelic self-sterile mutants, the cross being heterozygous with wild type at each of the two mutant loci. Obviously, this leads to the production of both sterile and self-fertile progeny. How, then, could this unbalanced heterothallism lead to balanced heterothallism, or is such an evolutionary step likely to occur at all? If the events just reviewed are considered in the light of recent discoveries regarding the nature of genetic material in both microorganisms and higher forms, such a step does not appear unlikely.

In the vast amount of literature available on the "biochemical genetics" of fungi, it has been repeatedly demonstrated that two mycelia of a fungus, differing only at two non-allelic mutant loci each of which blocks the synthesis of some necessary growth substance, can anastomose and grow normally as a heterokaryon on minimal medium lacking the two required growth substances. This is because each mutant locus is compensated for by the wild type allele of the other component of the heterokaryon. If the two biochemical mutants are crossed, the progeny will consist of the two mutant genotypes plus the double mutant and wild type autotrophic recombinants.

Our studies and those of Greis (1942) and Esser and Straub (1956) on sterility mutants of Sordaria have yielded comparable results. From the standpoint of growth-factor requirements our mutants are not like those described above, for they will grow on minimal medium. Many of them grow more slowly than normal, even on maximal medium, and are often brownish in color, unlike the wild type which is at first whitish and later develops darker tints apparently as the result of melanin formation. Nevertheless, a number of these cultures, when paired, produce fully fertile heterokaryotic mycelia that are wild type in appearance (figure 2). In one such cross, which has been studied more carefully than the others, each parent has a single major mutant locus blocking fertility. Since these loci are non-allelic, parental, double mutant, and wild type progeny are recovered, as in the case of the growth-factor mutants. This is a clear example of the unbalanced heterothallic behavior described above. It is obvious that a type of biochemical mutation is involved here. In each of the mutant strains a locus concerned with some phase of the sexual process has been affected so that the strain is rendered self-sterile. In the particular cross described here, one strain produces no ascogonia and therefore has its sexual development blocked at an earlier stage than does the other strain, which produces protoperithecia (and some sterile perithecia) when grown alone.

All of our matings of completely self-sterile mutants involving homoallelic combinations of major sterility loci have failed to cross or produce fertile heterokaryons, even when differences in other genetic factors were involved. Such results would be expected, just as it is not possible to obtain an autotrophic heterokaryon in Neurospora by attempting to combine two identical growth-factor mutants. Such a combination merely repeats and does not compensate for the particular deficiency. It is not likely, therefore, that a stable one-locus form of heterothallism can arise by the association of two identical sterility mutants. Also, it would be difficult to envision the repeated origin of balanced heterothallism in the fungi by means of a shift of one of the non-allelic loci to the same position as the other. There is, however, a more logical alternative, the essence of which is contained in the suggestion of Lewis (1954) that the action between mating-type loci represents "a complementary stimulant between unlike-gene products," and of Burnett (1956) that "the mating-type factors are of the nature of allelomorphic supergenes."

In recent years there has accumulated a considerable amount of information which demonstrates that many loci, some of which were formerly considered to be single genes, are actually groups of closely associated units that are also closely related in function. It has been further shown that these units are individually mutable and recombinable. The terms "cistron" and "muton" coined by Benzer (1957) in his genetic studies of phage probably have much in common with the compound locus and its subunits as discussed here.

The purple adenine mutants of *Neurospora crassa* (de Serres, (1956; Giles, *et al.*, 1957) will serve to illustrate the compound locus concept. At the *ad*-3 locus affecting adenine synthesis, 24 adenineless mutants were found to fall into two physiologically distinct groups, *ad*-3A and *ad*-3B. Heterokaryons between members of the two groups were adenine-independent, and inter-group crosses produced a small percentage of adenine-independent progeny by crossing over between units in the *ad*-3 region. Other examples substantiating the existence of compound loci in microorganisms are to be found in reports on genetics of phage (Benzer, 1957), bacteria (Demeric, 1956), and fungi, including yeast (Roman, 1956), Aspergillus (Pontecorvo *et al.*, 1953), and Neurospora (Bonner, 1951; Mitchell, 1955; St. Lawrence, 1956). In a number of instances involving the pairing of pseudoallelic genotypes in fungi, heterokaryons independent of the involved growth factor requirements fail to develop. For example, in *Aspergillus nidulans*, Pontecorvo, *et al.*, (1953) were able to identify three different biotinless mutants in the same region of the chromosome and closely linked to the *y*-locus in the order $y\ bi_2\ bi_3\ bi_1$. Although crosses between different pseudoallelic mutants gave a very small percentage of biotin-independent recombinants by means of crossovers within the compound locus (crossover frequency between $bi_2$ and $bi_1$ = 0.1 per cent), it was found that biotin independence fails to occur either in heterokaryons or in

diploids unless all three wild type units of the locus are assembled in order within the same chromosome. These authors speak of this biotin locus as a gene containing within it smaller units capable of mutation and recombination. Goldschmidt (1955), in a somewhat different interpretation, states that "multiple alleles are no longer different conditions of a single gene but changes in pattern within small sections of a chromosome which are not necessarily of the same extent but may overlap."

Although terminologies may differ, it is clear that there are small segments of chromosomes which are composed of several closely associated smaller units capable of mutation and recombination and in which the component units have similar functions and control related reactions. The literature thus far available on compound loci in fungi indicates that they generally contain from two to four mutable and recombinable units. In the absence of sufficient meiotic analyses to determine whether recombination among subunits occurs, a locus, though compound, would give the appearance of being a single unit or gene, in the conventional sense. As has been pointed out, while such loci may often be detected in fungi by the formation of phenotypically wild type heterokaryons, such heterokaryons do not always develop when pseudoallic mutants are paired.

There is reason to believe that the compatibility loci in fungi have properties similar to the compound loci controlling the growth of the organism, as described above. It has already been noted that matings homozygous for the same major sterility factor would not be expected to produce fertile perithecia, since each member of the pair would be deficient for the same step in sexual reproduction. However, if a particular locus controlling some essential phase of sexual reproduction should be composed of two or more mutable subunits, then it should be possible to have pseudoallelic mutations at this locus which would permit compatible crosses leading to the production of fertile perithecia. In view of the foregoing discussion of the biotin locus in *Aspergillus nidulans*, however, not all pseudoallelic crosses would be expected to result in fertility, since some of these loci might be able to function properly only when all wild type units are present in the same chromosome.

Since an undetermined number of separate loci appear to be involved in the control of sexual reproduction in a species, the chances of one-locus heterothallism originating in this manner should vary more or less in direct proportion to the number of such loci that are of a compound nature. It would also be a matter of chance, depending upon the occurrence and association of pseudoallelic mutants, as to which locus will control compatibility in the evolution of a heterothallic species. Also, the development of a heterothallic form does not preclude further mutations for sterility at other loci, for the latter have been actually observed in *Hypomyces solani* f. *cucurbitae* (Hansen and Snyder, 1943), *Cochliobolus heterostrophus* (Nelson, 1957), and probably *Dictyuchus monosporus* (Couch, 1926). This might also explain many instances of failure of formerly compatible strains

of heterothallic species to cross after extended periods of culture in the laboratory.

Evidence in support of the theory of a compound compatibility locus from genetic studies of homothallic species would naturally be difficult to produce at this time, in view of the fact that these fungi, until recently, have attracted little interest as subjects of genetic research. An observation of Greis (1942) may furnish an interesting lead, however. In studying a large number of self-sterile x-ray mutants of *Sordaria fimicola* he obtained a cross (I 11,923 × I 12,998) between a light mycelial culture with no ascogonia ("male") and a dark green ascogonial culture ("female") from which the asci produced four spores of each parental type, with segregation occurring in either the first or second meiotic division. He states that several asci were isolated and that all yielded similar progeny. In the text he gives analyses of only three. It is unfortunate that no further analysis of this cross was made, as this might have represented the first case of two compatible pseudoallelic mutants of a homothallic species being produced in the laboratory. At least this demonstrates a possible approach to the problem on an experimental basis.

The independent occurrence of two pseudoallelic mutations is by no means the only method by which the origin of one-locus heterothallism may be envisioned. It is also possible that a double mutation may occur at the same time among the units of a compound locus, the two mutated units finally becoming separated into homologous chromosomes through crossing over with the wild type locus in crosses. Since two pairs of spores in an ascus in which this event had occurred would contain the complementary pseudoallelic loci, the chances for their survival and early association is enhanced by their discharge, along with the other four spores, in a single spore mass from the ascus. Evidence for simultaneous double mutations in the same compound locus is rare, but there appears to be at least one demonstration of such an occurrence in the genetic studies of Saccharomyces by Roman (1956), who strongly supports "a view of the gene as a complex structure separable by mutation into individual elements." In his investigation of adenineless mutants, he found that the two units of the *Ad*5–*AD*7 locus could mutate simultaneously as well as independently. Since crosses of mutants *ad*5-*ad*7 × *ad*5 and *ad*5-*ad*7 × *ad*7 yielded only adenine-requiring diploids, while *ad*5 × *ad*7 yielded adenine independent diploids, Roman concluded that the two mutants were non-allelic but "tightly linked." The double mutants had not separated in the 163 asci analyzed, but this would not be surprising if the units were as closely linked as several that have been reported by other investigators.

There are other ways in which pseudoallelic sterility mutants might be produced in a homothallic species. Small deletions could achieve similar results. If such a deletion occurred within or adjacent to a compound locus essential to sexual reproduction in such a way as to include one or more (but not all) units of the locus, and if a complementary deletion occurred

at the other side of the locus in a homologous chromosome so as to remove one or more (but none of the same) units, the loci might then prove to be compatible and capable of initiating one-locus heterothallism. This, of course, would be dependent upon the occurrence of these events near enough in time and place for them to become effective. Minor deletions have been reported as occurring by means of unequal crossing-over (Laughnan, 1955), and they may be induced by various treatments, including irradiation. The most favorable deletion method that might lead to the establishment of true heterothallism would be the simultaneous occurrence, possibly as the result of ionization or some aberrant crossing over mechanism, of pseudoallelic deletions in homologous chromosomes at meiosis or in daughter chromatids during mitosis. Swanson (1957) states: "Since isochromatid deletions involve the breakage of two chromatids, it is thoroughly possible to have the breakage of both at the same time by a single path of ionization or of each individually by two different paths of ionization." If the path of ionization were at such an angle as to cause small pseudoallelic deletions (or unit mutations) in synapsed or recently split chromosomes, the opportunity for their early association in an effective heterothallic relationship would be increased.

*Coordinating phenomena.* Of primary interest in the present discussion would be the occurrence of any phenomena that would tend to bring together pseudoallelic sterility mutants to establish the heterothallic relationship. It has already been noted that in any common event at meiosis or mitosis that produced such mutants, their early association in sexual reproduction would be facilitated. But there remains the question of how sterility mutants that appear independently (probably the majority) could become associated in such a relationship. In Sordaria certain sterility mutants readily form fully fertile heterokaryons with the homothallic wild type (Olive, 1956) or with other non-allelic sterility mutants (Greis, 1942; Esser and Straub, 1956; Carr and Olive, in press). The heterokaryotic mycelia are wild type in appearance and often remain heterokaryotic in subsequent transfers. They produce crossed perithecia in abundance. We have found that in certain matings involving the wild type and a completely self-sterile mutant there is a vigorous response on the part of the wild type nuclei which, by means of anastomoses, pass over rapidly and in large numbers into the sterile mycelium, eventually converting it into a fertile one covered with perithecia, many of which are hybrid. If this attraction occurs in nature, it would explain how mutant loci for sterility could be carried along by the fungus until a complementary pseudoallelic mutation occurs, or, if the locus contains a two-unit mutation, until a crossover occurs in a heterozygous ascus, thus establishing one-locus heterothallism.

Disomic nuclei carrying a pair of homologous chromosomes, one with a mutant sterility locus and the other wild type at the same locus, could also be a means of perpetuating the mutant locus until a pseudoallelic sterility mechanism is established. Pseudo-wild type strains in Neurospora can

develop from ascospores containing disomic nuclei carrying a pair of homologous chromosomes with pseudoallelic, mutually complementary loci involving certain growth factor requirements (Mitchell, *et al.*, 1952; Pittenger, 1953). There is some evidence that the disomic relationship disassociates in the mycelium so that both n + 1 and n nuclei are present. There is no reason to believe that disomic nuclei carrying a pair of homologous chromosomes, one wild type and the other with a mutant sterility locus, could not occur in a homothallic species. In fact, such a phenomenon would be of more significance in species that are unable to form heterokaryotic mycelia by anastomosis.

A supporting argument in favor of the pseudoallelic, rather than strictly allelic, nature of the compatibility loci in heterothallic fungi may be derived from the observation that the two strains are sometimes unlike in some character other than compatibility but apparently controlled by the compatibility locus. For example, we have found that the *A* and *a* strains of *Ascobolus stercorarius* when grown separately may be fairly similar in appearance, but in matings fruiting bodies are produced more readily when oidia are transferred from *A* to *a* than when the direction is reversed. The *a* culture is stimulated to produce ascogonia more readily and more abundantly than is the *A* culture. One of the most striking examples is the morphological difference between paired male and female strains of *Ascosphaera apis*. One might postulate in this case that a mutation at a sex-controlling locus of a homothallic progenitor inhibited completion of the sexual process in the ascogonial strain (female) at a later stage and to a less extent than a pseudoallelic mutation in the other strain (male), which forms no sex organs at all. Comparative studies indicate that the fungus may have evolved from a homothallic form such as Monascus (Spiltoir, 1955).

## EVIDENCE FROM HETEROTHALLIC SPECIES

If the theory of pseudoallelic sterility factors is a feasible explanation of the origin of heterothallism, one might expect an extensive meiotic analysis of a heterothallic species to yield a small percentage of homothallic progeny by crossing over within the compatibility locus. In ascomycetes and lower fungi no studies have been sufficiently definitive to demonstrate conclusively that homothallic progeny may arise from a species showing balanced heterothallism. Numerous investigators have, from time to time, reported the occurrence of occasional self-fertile progeny in such species, but these have too often been discarded without further study under the category of contaminated cultures or mycelia derived from heterokaryotic ascospores, or simply as unexplainable. Bistis (1956) obtained a single-spore culture of *Ascobolus stercorarius* which was self-fertile, both *A* and *a* progeny being isolated from one of the resultant asci. The mycelium after several transfers became *a*. Nelson (1957) reports a self-fertile, single-ascospore culture of *Cochliobolus heterostrophus* which retains its fertility after repeated transfers, but whose ascospores produce

heterothallic progeny. In these and similar reports, there is insufficient evidence that these isolates represent reversions to true homothallism. They could be as readily attributed to ascospores that are heterokaryotic or that contain disomic nuclei carrying a homologous pair of compatibility chromosomes. The last possibility was suggested by St. Lawrence (personal communication), who obtained (1952) several single-ascospore cultures of *Neurospora crassa* which, although not self-fertile, could cross with either the *A* or *a* strain.

Evidence other than the occurrence of occasional self-fertile progeny should be looked for as indication of the complexity of the compatibility locus. Certain progeny of heterothallic species that can not be crossed with either parental mating type may have resulted from some alteration of the compatibility locus through mutation or crossing-over within the locus. Some of our single-spore isolates of *Ascobolus stercorarius* from nature have produced numerous irregular sterile bodies, and one produced apothecia of normal size in which paraphyses but no asci appeared (Yu, 1954). The condition was unstable and transfers eventually failed to produce these structures. No genetic study was made at the time to determine if this was a pleiotropic effect of the compatibility locus. Nelson (1957) obtained a single-spore isolate of *Cochliobolus heterostrophus* which produced perithecia with sterile asci. There is a possibility that such strains may develop as a result of crossing over within the compatibility locus. A complete tetrad analysis of asci from which such progeny are derived would throw much light upon this problem.

*Mechanisms preventing reversal of heterothallism.* There are several known genetic phenomena in fungi and other organisms that could explain why a reversal of heterothallism to homothallism would be unexpected in some species. On a chance basis alone, if compatibility pseudoalleles were no further apart than are the pseudoalleles of the two *p*-aminobenzoic acid-requiring strains of *Aspergillus nidulans* (Pontecorvo, *et al.*, 1953), recombination would occur in only one meiosis in 50,000. Also, if the heterothallic species has gone through a long period of evolution, it is most likely that the two pseudoalleles and their adjacent chromatin have evolved along at least slightly different lines, in which case any crossing over within the locus might be expected to produce alterations in the appearance and crossing ability of the progeny, possibly also sterility in matings, or even inviability of the progeny, but not self-fertility. In other words, neither pseudoallele may be able to function properly out of its own chromosomal environment. There is also the possibility that, in some species, crossing over within the compatibility locus occurs but the recombinant locus is unstable. Reported disturbances in mating reactions among yeast progeny (Mundkur, 1949) could have resulted from crossing over within the compatibility locus. A similar phenomenon might account for the "gene degredations" for loci controlling fermentation in yeast.

Garnjobst and Wilson (1956) have presented convincing evidence that the compatibility loci of *Neurospora crassa* cause protoplasmic incompatibility that tends to prevent hyphae of opposite mating types from anastomosing or from forming heterokaryons if anastomosis occurs. If there were also a repulsion at the locus during synapsis, it would act as a deterrent to crossing over within the locus. Such species would not be the most likely subjects for an investigation of reversion to homothallism. Species like *Ascophanus granulatus* (Gwynne-Vaughan and Williamson, 1930) and *Ceratostomella radicicola* (El-Ani, *et al.*, 1957), in which the two compatibility types appear readily capable of forming heterokaryons, should prove more amenable to such a study. If the results obtained by us and by Esser and Straub (1956) with non-allelic sterility mutants of *Sordaria* are indicative, the ability of the mating types to form fertile heterokaryons would be a less advanced form of heterothallism than that reported in *Neurospora crassa*.

*Pseudohomothallism.* Pseudohomothallic or miktohaplontic species are found both in ascomycetes and basidiomycetes. These produce meiospores that contain nuclei of both compatibility types and are therefore self-fertile. Obviously, from the genetic standpoint they belong to the heterothallic group. The question arises as to where they fit into the evolutionary picture. Dodge (1939) showed that a single mutation was sufficient to convert the normally 4-spored, asci of pseudohomothallic *Neurospora tetrasperma* into an 8-spored, typically heterothallic condition in crosses heterozygous for the mutant locus. Dodge, *et al.* (1950) suggest that this may indicate a reversal of one of the evolutionary steps by which *N. tetrasperma* arose from an 8-spored heterothallic species. This is a distinct possibility. On the other hand, there is at least a chance that the reverse is true. Whenever *A* and *a* are separated into different mycelia in *N. tetrasperma*, the nuclei readily reassociate themselves through anastomoses when compatible mycelia are paired. Unilateral nuclear migration frequently occurs, as we have also observed in our matings of certain self-sterile mutants of *Sordaria fimicola*, and the heterokaryon is maintained in subsequent transfers. During ascospore delimitation in *N. tetrasperma*, apparently as a result of a mutual attraction, the pairs of *A* and *a* nuclei cooperate in cutting out four spores (which does not occur in any of our Sordaria crosses). Not only do the investigations of Garnjobst and Wilson (1956) indicate just the opposite effects of the compatibility loci of 8-spored *N. crassa*, but they also show that two other loci, independent of the compatibility locus, act to interfere with heterokaryon formation in that species. In some ways, therefore, this appears to be a more modified and possibly more advanced form of heterothallism in Neurospora than is pseudohomothallism.

The position of the compatibility factor on the chromosome in the various species detracts from this concept. Genetic studies have shown that the compatibility loci of the three investigated species are at quite different crossover distances from the centromere, the locus being at the centromere in *N. tetrasperma* and at different distances from the centromere

in *N. crassa* and *N. sitophila*. Burnett (1956) states that the direction of evolution was probably from the 8-spored to the 4-spored form, in which case there would have developed a reduction in crossing over between mating type locus and centromere either by reduction in chiasma frequency or by deletion or inversion of the region between the two. Deletions of such an extent would probably have been lethal, but Burnett's alternative suggestions appear reasonable. Further study of the 4-spored species of several allied genera would probably shed more light upon this problem, as would careful analyses of interspecific crosses in Neurospora. Dodge (1928) has demonstrated that such crosses can be made, and, although much spore abortion occurs, some asci produce several mature spores. If the compatibility loci of these species were actually at physically different distances from the centromere and if crossing over were possible in interspecific crosses, one might expect to obtain *A* and *a* factors on the same chromosome in some of the progeny. No detailed study from this standpoint has yet been made.

*Multiple allele heterothallism.* There is probably no better evidence for the existence of complex compatibility loci in fungi than is to be found in the basidiomycetes. In a review of those groups (the majority) of club fungi in which multiple allele heterothallism has been reported, Whitehouse (1949) concludes that, of those species investigated, approximately 10 per cent are homothallic and 90 per cent heterothallic, with 35 per cent having bipolar and 55 per cent tetrapolar heterothallism. Whitehouse (1949, 1949 a), Papazian (1951), and Raper (1953, 1957) have presented convincing evidence that the compatibility (or "incompatibility") loci are complex ones composed of series of units. Papazian explains how the known genetic data in the tetrapolar *Schizophyllum commune* can be explained on the basis that there are about 10 units at each locus. Evidence of the pseudoallelic nature of these units is to be found in the comparatively frequent reports of "mutation" in compatibility, a phenomenon which Papazian and Raper believe results primarily from crossing over within the compatibility loci, and not from actual mutation. Both investigators note that the occurrence of the apparent "mutations" in pairs at meiosis is good reason to believe that they result from crossing over among the units of the locus. Raper estimates that crossing over in the *A* locus of Schizophyllum occurs at a rate of about 2–3 per cent to yield 'new' factors.

It has long been known that many compatibility types exist in a heterothallic hymenomycete. Single-basidiospore isolates are self-sterile but inter-group fertile with other single-spore isolates of the same fruiting body. In a bipolar species a single fruiting body produces only two compatibility types, while that of a tetrapolar species produces four. But when any single-spore isolate of a fruiting body from one locale is paired with any single-spore isolate of the same species from another locale, the chances are that they will cross, because of differences in the constitution of their compatibility loci, and produce fertile fruiting bodies. The compatibility loci, therefore, prevent selfing but at the same time permit a max-

imum of outbreeding. Papazian believes that the primary function of the compatibility loci ("incompatibility factors") is to "actuate some kind of 'trigger' mechanism while the fundamental factors controlling the processes leading to the formation of a fruiting-body are associated with other genes." This is probably the most logical explanation yet proposed to explain the function of these loci, which are clearly unlike those of lower fungi. Apparently, any difference between two allelic loci with respect to the units within them is sufficient to "trigger" the development of the dikaryon. The theory of mutual complementation by two loci, each containing a pseudallelic mutant component, would not be applicable here, although the origin of heterothallism in basidiomycetes was most likely by the same method as in lower fungi.

It is likely that the absence of sex organs in higher basidiomycetes has had much to do with the kind of development these loci have undergone. In the absence of sex organs, the compatibility mechanism has been reduced to one of controlling hyphal anastomosis and dikaryon formation, followed subsequently by karyogamy in the basidia. Both Papazian and Raper find that other factors in Schizophyllum, independent of the compatibility loci, affect dikaryotization and nuclear migration, a situation similar to that reported by Garnjobst and Wilson (1956) in *Neurospora crassa*.

The origin of the series of pseudalleles at the compatibility loci is at present a matter of conjecture. A phylogenetic approach would lead one to believe that the first appearance of heterothallism in basidiomycetes was of the type found in ascomycetes and that a heterothallic ascomycete was the ancestral form. This type is known to occur in the rust fungi, a primitive group in which sex organs of a type (spermatial organs or pycnia) are still produced. It would seem reasonable to assume that, in higher basidiomycetes, loci no longer involved in controlling the development of sex organs would be freer to evolve in other ways, now concerned primarily with dikaryotization. Such loci might have become complex ones by successive mutations occurring in the compatibility segment over a long period of time, along with crossing over within this region. Possibly a more plausible hypothesis, based on the concept of gene duplication, could be advanced. Laughnan (1955), in his studies of maize and with supporting data from reports on Drosophila, presents a case for the origin of clusters of similarly acting genes (pseudoalleles) by gene duplication. He believes that this duplication may occur by means of oblique synapsis and unequal crossing over. Duplication of the *bar* locus in Drosophila has actually been visually detected in a salivary gland chromosome. The two separable pseudoallelic units studied by Laughnan in maize have similar, though not exactly identical phenotypic effects.

In higher basidiomycetes, the units of the compatibility locus have a similar function – the control of dikaryotization – and their reapportionment by crossing over seems to have little or no phenotypic effect upon the progeny. Nonetheless, there is evidence from analyses of crosses and from the occurrence of certain "mutants" (intra-locus recombinants) more fre-

quently than others that the units are not exactly identical or do not have precisely the same relationship within the locus.

It would be appropriate to mention here the reports of occasional spontaneous dikaryotization in these fungi. This phenomenon might be the result of errors in duplication or of uneven mitotic crossing over in a compatibility locus during nuclear divisions in the mycelium, thus giving rise to "new" mating types. Also it is possible that some single-spore cultures, which at first appear homokaryotic, later become dikaryotic as a result of dissociation of homologous compatibility chromosomes carried by disomic nuclei.

Whitehouse (1950) believes that the major factor in the evolution of the angiosperms was the appearance of multiple-allele heterothallism, and he concludes that the character has arisen very seldom in evolutionary history. It may be that is has originated only once (followed later by the appearance of tetrapolarity) in the basidiomycetes, although no comparative cytogenetic studies are available that would support such a conclusion at this time. If this were true, however, Raper's conclusion that the different patterns of sexuality in this group are "probably the result of a continuous process of change from tetrapolarity to bipolarity to homothallism" would not be unreasonable, in view of the distribution and proportion of these three types of sexuality in the higher basidiomycetes. The evidence suggests strongly that the homothallic species have generally evolved from heterothallic ones. For example, in a number of species characterized by tetrapolar heterothallism, homokaryotic strains have been obtained that produce fruiting bodies when grown alone and in whose basidia like nuclei fuse and undergo meiosis. This switch from heterothallism to homothallism is not surprising in view of the fact that the compatibility loci of these fungi participate in the sexual process at a less involved level than in lower fungi.

This paper is by no means to be considered an exhaustive attempt to explain all the ways in which heterothallism may have arisen and evolved in the fungi. The present proposals are made in the light of recent discoveries of genetic behavior both in microorganisms and higher forms, and the main purpose of the paper will have been accomplished if it in any way stimulates renewed interest in the evolution of sexual processes in organisms, with the resultant contribution of new evidence bearing upon this problem.

## SUMMARY

Three major types of life cycles in the fungi—homothallism, unbalanced heterothallism, and balanced heterothallism—and their possible evolutionary relationships to each other are discussed in the light of recent genetic discoveries in microorganisms and higher forms. It is concluded that the most likely explanation of the origin of one-locus two-allelic heterothallism in the fungi is to be found in the compound-locus hypothesis. This would

involve the occurrence – independently or simultaneously – and association of pseudoallelic self-sterility mutations in a compound locus (of two or more subunits). It is proposed that the compound nature of the multiple-allele compatibility loci of bipolar and tetrapolar higher basidiomycetes may have resulted from successive duplications during evolution.

## LITERATURE CITED


Benzer, S., 1957, The elementary units of heredity. *In* Symposium on the Chemical Basis of Heredity, pp. 70-93

Bistis, G., 1956, Studies on the genetics of *Ascobolus stercorarius* (Bull.) Schröt. Bull. Torrey Bot. Club 83: 35-61.

Blakeslee, A. F. 1904, Sexual reproduction in the Mucorineae. Proc. Amer. Acad. Arts & Sci. 40: 205-319.

Bonner, D. M., 1951, Gene-enzyme relationships in *Neurospora.* Cold Spring Harbor Sympos. Quant. Biol. 16: 143-154.

Burnett, J. H., 1956, The mating systems of fungi I. New Phytologist 55: 50-90.

Chilton, S. J. P., G. B. Lucas, and C. W. Edgerton, 1945, Genetics of Glomerella. III. Crosses with a conidial strain. Amer. Jour. Bot. 32: 549-554.

Couch, J. N., 1926, Heterothallism in Dictyuchus, a genus of water moulds. Ann. Bot. 40: 848-881.

Demerec, M., 1956, A comparative study of certain gene loci in Salmonella. Cold Spring Harbor Sympos. Quant. Biol. 21: 113-121.

de Serres, F. J., 1956, Studies with purple adenine mutants in *Neurospora crassa.* I. Structural and functional complexity in the *ad*-3 region. Genetics 41: 668-676.

Dodge, B. O., 1928, Production of fertile hybrids in the ascomycete Neurospora. Jour. Agric. Res. 36: 1-14.

1939, A new dominant lethal in Neurospora. The E locus in *N. tetrasperma.* Jour. Heredity 30: 467-474.

J. R. Singleton, and Anita Rolnick, 1950, Studies on Lethal E gene in *Neurospora tetrasperma,* including chromosome counts also in races of *N. sitophila.* Proc. Amer. Phil. Soc. 94: 38-52.

Edgerton, C. W., 1912, Plus and minus strains in an ascomycete. (Abstract). Science 35: 151.

El-Ani, A. S., L. J. Klotz, and W. D. Wilson, 1957, Heterothallism, heterokaryosis, and inheritance of brown perithecia in *Ceratostomella radicicola.* Mycologia 49: 181-187.

Emerson, R., 1950, Current trends of experimental research on the aquatic phycomycetes. Ann. Rev. Microbiol. 4: 169-200.

Esser, K., and J. Straub, 1956, Fertilität im Heterocaryon aus zwei sterilen Mutanten von *Sordaria macrospora* Auersw. Zeit. f. indukt. Abstammungs-u. Vererbungsl. 87: 625-626.

Frandsen, K. J., 1946, Studier over *Sclerotinia trifoliorum* Eriksson. 220 pp.

Garnjobst, Laura, and J. F. Wilson, 1956, Heterocaryosis and protoplasmic incompatibility in *Neurospora crassa.* Proc. Nat. Acad. Sci. 42: 613-618.

Giles, N. H., F. J. de Serres, and Evelyn Barbour, 1957, Studies with purple adenine mutants in *Neurospora crassa.* II. Tetrad analyses from a cross of an *ad*-3A mutant with an *ad*-3B mutant. Genetics 42: 608-617.

Goldschmidt, R. B., 1955, Theoretical Genetics. Univ. Cal. Press. 563 pp.

Greis, H., 1942, Mutations-und Isolationsversuche zur Beinflussung des Geschlechtes von *Sordaria fimicola* (Rob.). Zeit. f. Bot. 37: 1-116.

Hansen, H. N., and W. C. Snyder, 1946, Inheritance of sex in fungi. Proc. Nat. Acad. Sci. 32: 272-273.

Hammons, L. M., G. Pontecorvo, and A. W. S. Bufton, 1952, Perithecium analysis in *Aspergillus nidulans.* Heredity 6: 135.

Laughnan, J. R., 1955, Structural and functional bases for the action of the *A* alleles in maize. Amer. Nat. 89: 91-104.

Lewis, D., 1954, Comparative incompatibility in Angiosperms and fungi. Adv. In Gen. 6: 235-285.

Mitchell, M. B., 1955, Aberrant recombination of pyridoxine mutants of Neurospora. Proc. Nat. Acad. Sci. 41: 215-220.

T. H. Pittenger, and H. K. Mitchell, 1952, Pseudo-wild types in *Neurospora crassa*. Proc. Nat. Acad. Sci. 38: 569-580.

Mundkur, B. D., 1949, Evidence excluding mutations, polysomy, and polyploidy as possible causes of non-Mendelian segregations in Saccharomyces. Ann. Missouri Bot. Gard. 36: 259-280.

Nelson, R. R., 1957, A major gene locus for compatibility in *Cochliobolus heterostrophus*. Phytopath. 47: 742-743.

Olive, L. S., 1956, Genetics of *Sordaria fimicola*. I. Ascospore color mutants. Amer. Jour. Bot. 43: 97-107.

Papazian, H. P., 1951, The incompatibility factors and a related gene in *Schizophyllum commune*. Genetics 36: 441-459.

Pittenger, T. H., 1953, The general incidence of pseudo-wild types in *Neurospora crassa*. Genetics 39: 326-342.

Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. MacDonald, and A. W. S. Bufton, 1953, The genetics of *Aspergillus nidulans*. Adv. in Gen. 5: 141-238.

Raper, J. R., 1953, Tetrapolar sexuality. Quant. Rev. Biol. 28:233-259.

1957, Hormones and sexuality in lower plants. *In* Symposia Soc. Exper. Biol. 11: 143-165.

Roman, H., 1956, Studies of gene mutation in *Saccharomyces*. Cold Spring Harbor Sympos. Quant. Biol. 21: 175-185.

St. Lawrence, Patricia, 1952, The association of particular linkage groups with their respective chromosomes in *Neurospora crassa*. Ph.D. Thesis, Columbia University.

1956, The *q*-locus of *Neurospora crassa*. Proc. Nat. Acad. Sci. 42: 189-194.

Spiltoir, C. F., 1955, Life cycle of *Ascosphaera apis (Pericystis apis)*. Amer. Jour. Bot. 42: 501-508.

and L. S. Olive, 1955, A reclassification of the genus Pericystis Betts. Mycologia 47: 238-244.

Swanson, C. P., 1957, Cytology and Cytogenetics. 596 pp. Prentice-Hall.

Wheeler, H. E., 1954, Genetics and the evolution of heterothallism in Glomerella. Phytopath. 44: 342-345.

Whitehouse, H. L. K., 1949, Multiple-allelomorph heterothallism in the fungi. New Phytol. 48: 212-244.

1949 a, Heterothallism and sex in the fungi. Cambr. Phil. Soc. Biol. Rev. 24: 411-447.

1950, Multiple-allelomorph incompatibility of pollen and style in the evolution of the Angiosperms. Ann. Bot. (n.s.) 14: 119-216.

Yu, Clare, 1954, The Culture and spore germination of *Ascobolus* with emphasis on *A. magnificus*. Amer. Jour. Bot. 41: 21-30.

Vol. XCII, No. 865 The American Naturalist July–August, 1958

# LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

## AN INTRAFERTILE COLONY OF PARAMECIUM BURSARIA*

The genetically effective population size of a species which forms local colonies is a function of the exchange of individuals between colonies (migration) and of the density of the breeding individuals in a continuously inhabited niche. As the writer has pointed out (Siegel, 1956), the existence of binary mating types in some species as opposed to multiple mating types in other closely related species may serve to influence the genetically effective density of a local population. *Paramecium aurelia* consists of a group of reproductively isolated varieties or "syngens" (Sonneborn, 1957) within each of which only two intercompatible mating types occur. The varieties of *P. bursaria* embrace two to eight mating types. Systems of multiple mating types, as contrasted with systems of binary mating types would be expected to enhance the probabilities for conjugations, hence genetic recombinations, because the chances for encounters between cells of complementary mating types are improved. Whatever the biological significance of systems of binary and multiple mating types (see Sonneborn, 1957, for a discussion of contrasting interpretations), some justification for the assumption that conjugation in *P. bursaria* is more than a laboratory phenomenon is in order. In view of these considerations it is important to record the discovery of a localized colony of *P. bursaria* in which all four of the possible mating types for variety 1 are represented.

On January 28, 1958, seven samples of pond water were collected from various sites along the shore of Malibu Lake, Malibu, California. Each sample contained about 150 ml. of water and organic debris. An innoculum of *Aerobacter cloacae* was added to each sample to provide a suitable food organism for any individuals of *P. bursaria* which might have been present. Two days later, two of the samples were found to contain individuals identified as *P. bursaria*; the other five samples contained no *P. bursaria* on that day or later and were discarded. On the afternoon of January 30, 34 cells which had begun to form 17 mating pairs were isolated from one of the positive samples into a baked lettuce infusion; these cells, in early stages of conjugation, were forced to separate before they could complete conjugation by repeatedly expelling them from a micropipette. In this way, 33 sexually mature vegetative lines were obtained; one cell died, probably as a result of handling. Cells which had not found mates were present in

*This investigation was supported by a grant from the National Science Foundation.

the culture but were not isolated. The second positive sample was not studied further; in this case the population was relatively very small and conjugation stages were not noticed.

Mass cultures of the above 33 lines were established and samples from each were mixed in various combinations to reveal the mating types present. Four intrasterile but intercompatible groups of cultures, each group representing one mating type, were present. I am indebted to Dr. T. T. Chen who kindly identified the mating types. The frequency of each mating type was as follows: mating type A, 13 lines; mating type B, 16 lines; mating type C, 1 line; mating type D, 3 lines. It should be emphasized that all of the interfertile mating types known for variety 1 (see Chen, 1946) were present in this single sample of pond water. Conjugations among the present clones have been induced and all crosses so far studied yield high proportions of vigorous F1 clones which can be raised to sexual maturity in two to three weeks.

In view of the facts that accurate population counts were not made immediately after obtaining the samples from nature and that enrichment was encouraged by the addition of food to the samples before the original populations were observed, estimates of density of the individuals in nature should not be attempted. This does not detract from the important conclusion that *P. bursaria* has been found to exist in isolated colonies within which sexually mature individuals of complementary mating types occur. Pringle (1956) was able to show that a local population of *P. aurelia* consisted of heterozygous (exconjugant) as well as homozygous clones; similar techniques could be used to reveal exconjugants among clones of *P. bursaria* collected from nature. Furthermore, since conjugation invariably results in the production of new clones which are initially sexually immature, the fact of conjugation in nature could be established through the isolation of immature individuals from a wild population.

## LITERATURE CITED

Chen, T. T., 1946, Varieties and mating types in *Paramecium bursaria*. 1. New variety and types, from England, Ireland, and Czechoslovakia. Proc. Nat. Acad. Sci. 32: 173-181.

Pringle, C. R., 1956, Antigenic variation in *Paramecium aurelia*, variety 9. Z. Abst. Vererbls. 87: 421-430.

Siegel, R. W., 1956, Mating types in Oxytricha and the significance of mating systems in Ciliates. Biol. Bull. 110: 352-357.

Sonneborn, T. M., 1957, Breeding systems, reproductive methods, and species problems in Protozoa, in the Species Problem. pp 155-324. AAAS, Washington.

R. W. SIEGEL

Department of Zoology
University of California
Los Angeles, California
March 27, 1958

## SELECTION FOR INCREASED RECOMBINATION IN DROSOPHILA MELANOGASTER

It has been known for many years that recombination varies according to environmental and genetic factors. Recently, however, definite evidence of variation in recombination when there is selection of recombinants between closely linked markers has been found in *Aspergillus nidulans* by Pritchard (1955) and Calef (1957). The increase in recombination frequencies was greatest near the point of selection and decreased with distance from it. The closer the linkage between the markers, the greater was the increase. In *Drosophila pseudoobscura*, Levine and Levine (1955) showed that various strains differed in amounts of crossing over thus suggesting genetic control. Early selection experiments in *D. melanogaster* of Gowen (1919) and Detelfsen and Roberts (1921) were either ineffective or inconclusive.

TABLE 1

RECOMBINATION BEFORE AND AFTER SELECTION

USING MATING $\frac{b\ pr\ vg}{+\ +\ +} = \frac{b\ pr\ vg}{b\ pr\ vg}$

| | *b pr vg* | *+++* | *b++* | *+ pr vg* | *b pr+* | *++ vg* | *b+ vg* | *+ pr+* | T |
|---|---|---|---|---|---|---|---|---|---|
| Before selection | 351 | 374 | 19 | 15 | 33 | 33 | 3 | 4 | 832 |
| After selection | 354 | 420 | 28 | 30 | 21 | 33 | 3 | 5 | 894 |

Recombination Values

| Segment | Before selection | After selection | $\chi_1^2$ for difference |
|---|---|---|---|
| *b–pr* | 4.93 ± .75 | 7.38 ± .87 | 4.05 |
| *pr–vg* | 8.77 ± .98 | 6.94 ± .85 | 1.77 |
| *b–vg* | 12.02 ± 1.13 | 12.53 ± 1.11 | .06 |

In table 1 the results of selection for crossovers between the closely linked markers black (*b*) and purple (*pr*) in *D. melanogaster* are given. These two genes are situated near the centromere where genetic and environmental factors often have their maximum effect. A third gene vestigial (*vg*) is also in the stock. Crossovers between *b* and *pr* could be selected in two out of three matings, the third being a preparatory mating:

(1) $\frac{b\ pr\ vg}{+\ +\ +} = \frac{b\ pr\ vg}{b\ pr\ vg}$

(2) $\frac{b\ +\ +}{b\ pr\ vg} = \frac{+\ pr\ vg}{b\ pr\ vg}$

(3) $\frac{b\ +\ +}{+\ pr\ vg} = \frac{b\ pr\ vg}{b\ pr\ vg}$

mating (1)

Recombination values are given with $\chi_1^2$ values testing the significance of the differences before and after selection for nine generations. The results show, as expected, a significant *b-pr* increase ($P < .05$). There is an insignificant *pr-vg* decrease probably as a result of a selection for non-recombinants which necessarily must result from the mating system used.

These results confirm those of Pritchard and Calef who, however, obtained a far greater effect which spread into the neighboring segments. This is probably due to the very small distance over which selection was practiced, the distances in the experiment reported here being much greater.

## LITERATURE CITED

Calef, E., 1957, Effect on linkage maps of selection of cross-overs between closely linked markers. Heredity 11: 265–279.

Detelfsen, J. A. and E. Roberts, 1921, Studies on crossing-over. I. The effect of selection on cross-over values. J. Exp. Zool. 32: 332–351.

Gowen, J. W., 1919, A biometrical study of crossing-over. Genetics 4: 205–250.

Levine, R. P. and E. E. Levine, 1954, The genotypic control of crossing-over in *Drosophila pseudo-obscura*. Genetics 39: 677–691.

Pritchard, R. H., 1955, The linear arrangement of a series of alleles of *Aspergillus nidulans*. Heredity 9: 343–371.

P. A. PARSONS

DEPARTMENT OF GENETICS
UNIVERSITY OF CAMBRIDGE
May 10, 1958